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Part 1: A Study of the Hydrolysis and Oxygen-18 Exchange of Oxygen and Thiol Esters in Basic Media.

Part 2: An Investigation of the Reactivity of Bifunctional Thiol Carboxylic Acids Towards a Distorted Amide.

by

Brenda Ann Kellogg

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

Fall 1995

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FACULTY OF GRADUATE STUDIES AND RESEARCH

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ABSTRACT

Part 1- The hydrolysis of phthalide and thiophthalide have been studied in highly alkaline media, T = 25°C, μ = 3.0 (KCl). For both esters an upward curvature in a plot of k_{hyd} vs. [OH^-] is observed suggestive of the onset of a second order in [OH^-] process in the hydrolysis. However carbonyl ^{18}O exchange studies and solvent kinetic isotope effect studies suggest that curvature is not due to the involvement of two hydroxides in the hydrolytic process. The most likely explanation for the curvature is an incorrect correlation of k_{hyd} with [OH^-] in this highly basic medium. A reinvestigation of the alkaline hydrolysis of methyl *o*-methoxybenzoate accompanied by ^{18}O-exchange studies failed to detect unambiguous evidence for a *bona fide* second order in [OH^-] process.

Solvent kinetic isotope effect studies have been carried out on the hydrolysis and ^{18}O exchange of the esters ethyl toluoate and *iso*propyl toluoate in alkaline H_2O and D_2O media to probe the question of whether the anionic tetrahedral intermediates produced during the base hydrolysis of esters are in protonic equilibrium. The observation of solvent kinetic isotope effects near unity ($(k)_{H_2O/D_2O} \leq 1$) for both k_{ex} and the ratio k_{ex}/k_{hyd} indicate that the oxygens of the tetrahedral intermediate are protonically equilibrated.

Part 2 - A number of bifunctional thiol-acids have been studied as potential catalysts for the hydrolysis of distorted amide **1**. It was found that thiol-acids **2**, **5**, and **6** are effective agents for the ring opening of amide **1**. The pH-rate data for these thiol acids indicate that the two predominant modes of reaction with **1** involve attack of the monoanionic form of the thiol acid on the neutral amide and attack of the monoanion on the protonated amide. The mechanism proposed here for the reaction of thiol acids **2**, **5**,

and **6** with neutral **1** involves both a nucleophilic group and an acidic functionality for the trapping of the tetrahedral intermediate. Such a mechanism is entirely consistent with mechanisms postulated previously for the reactions of dicarboxylic acids, β-amino alcohols and ammonium thiolates with **1**.

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LIST OF ABBREVIATIONS

a_i	activity of species i
Asp	aspartic acid
α-CD	alpha cyclodextrin
α_i	fraction of material in the form of species i
β-CD	beta cyclodextrin
CPA	carboxypeptidase A
Cys	cysteine
DKIE	deuterium kinetic isotope effect
ε	molar extinction coefficient
f_i	activity coefficient of species i
Glu	glutamic acid
HRMS	high resolution mass spectroscopy
ip	ion pair
k_{ex}	pseudo first order rate constant for exchange
k_{hyd}	pseudo first order rate constant for hydrolysis
k_2^{obs}	pH dependent overall second order rate constant
M^+	molecular ion peak
NLLSQ	nonlinear least squares
pNPA	para-nitrophenyl acetate
SKIE	solvent kinetic isotope effect
To^-	anionic tetrahedral intermediate
To^{-2}	dianionic tetrahedral intermediate
$T_{(OH)_2}$	neutral tetrahedral intermediate
$T^{\pm}$ or T_{zw}	zwitterionic tetrahedral intermediate
Tyr	tyrosine
μ	ionic strength

Part 1 - Chapter 1

The Hydrolysis of Phthalide, Thiophthalide and Methyl *o*-Methoxybenzoate in Highly Alkaline Media*

Introduction

(A) Mechanism of the Alkaline Hydrolysis of Carboxylic Esters.

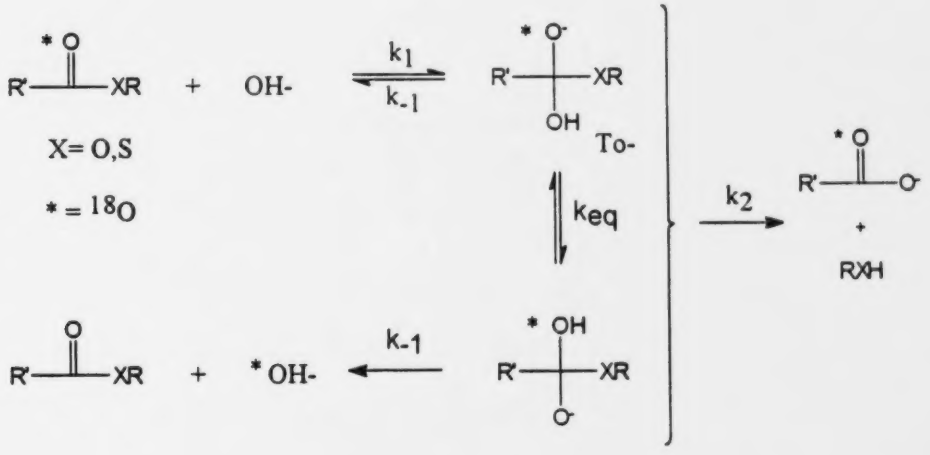
The alkaline hydrolysis of carboxylic esters is one of the most well studied reactions in organic chemistry and as a result many of the details of the mechanism are well established. In 1934 Polanyi and Szabo[1] showed that the hydrolysis of simple carboxylic esters in base solution proceeds with acyl oxygen fission by showing that hydrolysis of esters in $Na^{18}OH$ / $H_2{}^{18}O$ results in formation of ^{18}O labelled carboxylic acid. Day and Ingold[2] proposed that the alkaline hydrolysis of esters may proceed through either a tetrahedral intermediate (addition-elimination mechanism) or a tetrahedral transition state (direct substitution mechanism). Bender[3] was the first to offer convincing evidence for the existence of a tetrahedral intermediate on the hydrolysis pathway. He did this by subjecting ester labelled with ^{18}O in the carbonyl group to the hydrolysis conditions. Unreacted ester was recovered from the medium after various periods of partial hydrolysis and examined for loss of ^{18}O in the carbonyl O. If a tetrahedral addition intermediate was formed reversibly (**Scheme 1**) then recovered ester would be expected to

---

* A version of this chapter has been published. Kellogg, B.A.; Brown, R.S.; McDonald, R.S. *J. Org. Chem.* **1994**, *59*, 4652.

contain less ^{18}O than the starting ester. (This statement is based on the assumption that proton transfer between the 2 oxygens of the tetrahedral intermediate, required to allow expulsion of $^{18}OH^-$, is very rapid compared to breakdown of the tetrahedral intermediate, i.e. $k_{eq} >> (k_{-1} + k_2)$.)

Scheme 1:



If the reaction instead proceeds through a tetrahedral transition state then there should be no opportunity for loss of ^{18}O label from the ester during the course of its hydrolysis. In this case the ^{18}O content of recovered ester should be identical to that of starting material.

Bender[3] found that both ethyl and *iso*propyl benzoate exhibited ^{18}O exchange concurrent with hydrolysis in H_2O (25°C) with k_{ex}/k_{hyd} = 0.21 and 0.37 respectively, where:

(1) $$k_{ex} = \frac{k_1 k_{-1}[OH^-]}{2(k_{-1} + k_2)}$$ = pseudo first order rate constant for exchange

(2) $$k_{hyd} = \frac{k_1 k_2[OH^-]}{k_{-1} + k_2}$$ = pseudo first order rate constant for hydrolysis

and

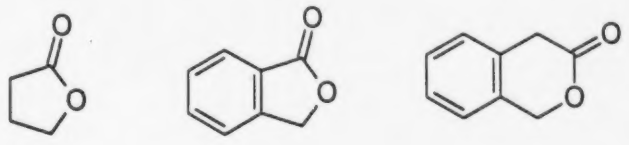
(3) $k_{ex}/k_{hyd} = k_{-1}/2k_2$

(These equations are derived below.) The ratio k_{ex}/k_{hyd} is therefore a measure of the relative partitioning of the tetrahedral intermediate back to starting material or on to products[4].

Several other studies involving application of ^{18}O exchange methodology to the alkaline hydrolysis of esters were carried out by Bender and others[5], following this initial investigation. It is important to note here that findings reported in a 1968 paper by Shain and Kirsch[6] contradicted many of the ^{18}O exchange results obtained by Bender and coworkers in earlier studies. The results of Shain and Kirsch might be anticipated to be correct because of the higher incorporation of ^{18}O label used in their studies and the more careful experimental work. Shain and Kirsch, in contrast to Bender and Thomas[5c], found no ^{18}O exchange out of labelled methyl benzoate, methyl *p*-nitrobenzoate or methyl *p*-aminobenzoate during alkaline hydrolysis in 33% dioxane/ H_2O. However methyl benzoate and ethyl benzoate both exhibited exchange during basic hydrolysis in pure water (k_{ex}/k_{hyd} = 0.036 (methyl benzoate), k_{ex}/k_{hyd} = 0.079 (ethyl benzoate), 25°C)[6]. A comparison of the partitioning ratio of methyl and ethyl benzoate in water indicates that this ratio decreases as the basicity of the leaving group decreases (or as apparent leaving group ablility increases) as expected if $k_{ex}/k_{hyd} = k_{-1}/2k_2$. Furthermore it appears that the amount of exchange exhibited by a given ester increases as the polarity of the medium increases. The most likely explanation for this observation is that a solvent of higher dielectric constant

more effectively solvates a departing HO^- than a departing RO^-, thus favouring partitioning of the tetrahedral intermediate towards starting materials.

A number of studies have also been carried out on the ^{18}O exchange of lactones in alkaline media. Deslongchamps has argued[7] that small ring lactones which are constrained to adopt the E configuration will never exhibit ^{18}O exchange irrespective of the relative sizes of k_2 and k_{-1}, because expulsion of $^{18}OH^-$ from the first formed tetrahedral intermediate is stereoelectronically forbidden. In apparent support of this theory no ^{18}O exchange in the basic hydrolysis of lactones **1-3** was observed[8].



1 **2** **3**

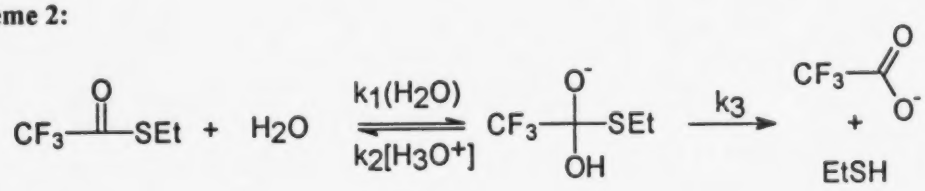
However convincing evidence demonstrating that stereoelectronic control is not important in governing the direction of breakdown of anionic tetrahedral intermediates has been provided by Brown and coworkers[9] and by Perrin and Nunez[10] in studies involving the basic hydrolysis of toluamides and amidines respectively. Additional evidence arguing against Deslongchamps interpretation of the lack of ^{18}O exchange in lactones was provided in a study by Hillery and Cohen[11] which showed that various methyl substituted phthalides exhibited substantial ^{18}O exchange during hydrolysis in base. It therefore seems most likely that the lack of ^{18}O exchange in lactones **1-3** can be explained simply in terms of a mechanism involving rate limiting attack of OH^- (i.e. $k_2 >> k_{-1}$).

(B) Assumptions Involved in the Use of ^{18}O Exchange Mehodology.

Several important assumptions are involved in the use of ^{18}O exchange methodology. These are considered below:

Assumption #1). *Oxygen-18 exchange out of the ester carbonyl occurs via an intermediate lying on the hydrolysis pathway.* Although this assumption seems entirely reasonable, it is nonetheless conceivable that exchange could occur by some process independent of the hydrolysis reaction. Bender and Heck[12] were the first to provide convincing kinetic evidence that the intermediate leading to exchange is the same intermediate formed during the hydrolysis reaction. This proof was based on the results of a kinetic study by Bruice and Fedor[13] which demonstrated the formation of an intermediate during the neutral hydrolysis of ethyl trifluorothiolacetate. The pH-rate profile for this thiol ester in the range pH 2-6 can be explained by the mechanism shown in **Scheme 2** involving H_2O attack on the thiol ester catalyzed by a second molecule of water acting as a general base. The tetrahedral intermediate thus formed breaks down in an unsymmetrical fashion, either by general acid catalyzed expulsion of OH by H_3O^+ or by unassisted expulsion of thiolate.

Scheme 2:



The pseudo first order rate constant for disappearance of ester according to this process is given in equation **(4)**.

(4) $$k_{hyd} = \frac{k_1 k_3}{k_3 + k_2[H^+]}$$

(5) or $$\frac{1}{k_{hyd}} = \frac{1}{k_1} + \frac{k_2[H^+]}{k_1 k_3}$$

Thus from a plot of $1/k_{hyd}$ vs. $[H^+]$, Fedor and Bruice were able to obtain values for k_1 and the partitioning ratio, k_2/k_3. If the tetrahedral intermediate in this scheme is assumed to be the same intermediate responsible for ^{18}O exchange out of labelled ester then the expression $k_{ex}/k_{hyd} = k_2[H^+]/2k_3$ can be derived. Thus the value of the ratio k_{ex}/k_{hyd} at a particular pH can be predicted based on the rate constants determined in the hydrolysis study. When Bender and Heck carried out the ^{18}O exchange experiments with labelled thiolester, they found that the experimentally determined ratio, k_{ex}/k_{hyd}, was identical to that predicted from the partitioning ratio, k_2/k_3, obtained from the Bruice and Fedor study. This demonstrated unequivocally that the intermediate leading to exchange is the same tetrahedral intermediate that lies on the hydrolysis pathway.

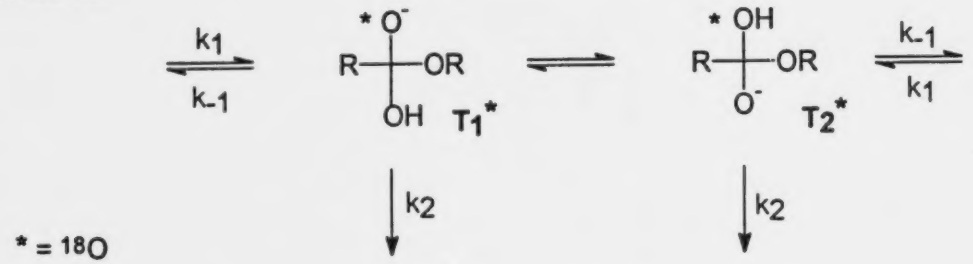
In a more recent paper Brown and coworkers[9] have used a similar type of analysis to show that the intermediate leading to exchange in the basic hydrolysis of the amide N-toluoylpyrrole, is the same intermediate that lies on the hydrolysis pathway.

Assumption #2). *The oxygens of the tetrahedral intermediate (To^-) are protonically equilibrated so that there is equal probability of expelling $^{16}OH^-$ or $^{18}OH^-$ upon reversal of To^- to starting material.* This assumption requires that $k_{eq} >> (k_{-1} + k_2)$ (**Scheme 1**). Only if this condition is met can the ratio k_{ex}/k_{hyd} be equated to $k_{-1}/2k_2$ thus permitting identification of the rate limiting step in the hydrolysis reaction. There has been much debate in the literature as to the validity of this assumption, however the issue has

remained unresolved until recently[14]. This problem will be discussed in greater detail in Chapter 2 which describes isotope effect studies designed to probe the identity of the rate limiting step for exchange.

Assumption #3). *The oxygen kinetic isotope effect on the formation and breakdown of tetrahedral intermediates T_1^* and T_2^** **(Scheme 3)** *is small, so that there is no significant difference in the rates of cleavage of C-^{16}O and C-^{18}O bonds.*

Scheme 3:



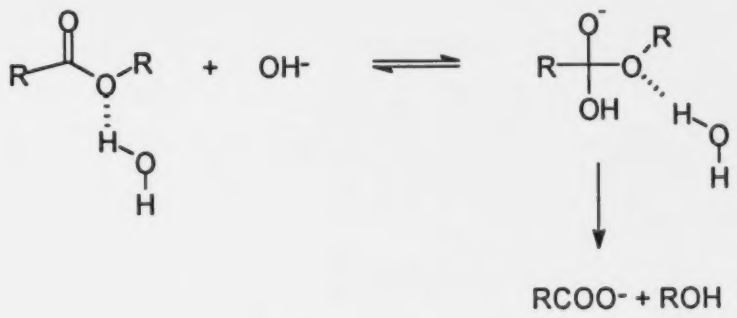
This assumption is undoubtedly valid since kinetic isotope effects involving heavy atoms are known to be small due to the small difference in zero point energies of bond vibrations involving the light and heavy isotope[15]. Measured isotope effects[16] involving $^{16}O/^{18}O$ usually fall in the range of 1-6% (i.e. $k^{16_o} / k^{18_o} = 1.01\text{-}1.06$) so that any isotope effect on k_{ex} would presumably be undetectable over normal experimental error.

(C) Mechanism of the Alkaline Hydrolysis of Thiol Esters.

The alkaline hydrolysis of thiol esters has also been extensively investigated[17] and the mechanism appears to similar to that for the O ester analogues. These studies have shown that the rates of alkaline hydrolysis of thiol esters can be either larger or smaller than the rates of hydrolysis of the corresponding O esters, although the difference is usually small (within a factor of two). Notably, although the rates are very similar, the

activation parameters differ significantly. It was consistently found that activation enthalpies are less favourable, but activation entropies more favourable, for thiol ester hydrolysis as compared to oxygen ester hydrolysis. To explain this observation, Schaefgen[17a] has suggested that addition of OH^- to the ester carbonyl is aided in O esters by a H bonding interaction betwen the alkyl O and H_2O (**Scheme 4**).

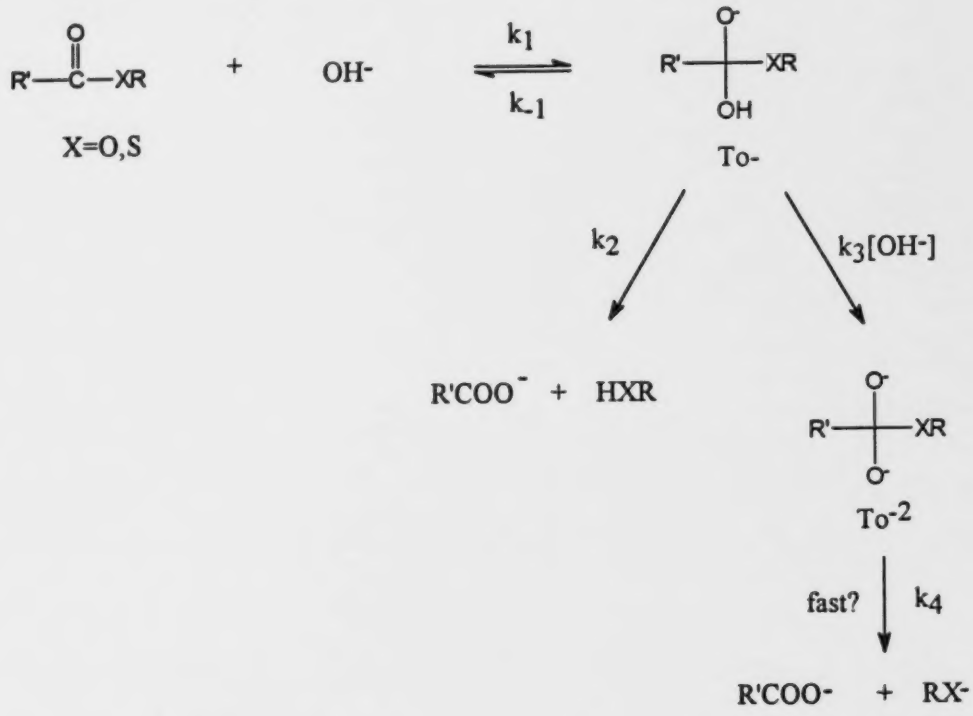
Scheme 4:



This interaction is not expected to be important in thiol esters because of the poor hydrogen bonding capacity of sulfur[18]. Such an interaction could help to explain why thiol esters and oxygen esters appear to be equally susceptible to attack by OH^- even though it is often claimed that resonance stabilization of thiol esters is less effective than for O esters[19]. However, any explanation of the relative reactivities of S and O esters towards OH^- really requires a knowledge of the rate limiting step for both processes. Although a large amount of ^{18}O exchange work has been carried out with O esters allowing identification of the rate limiting step for a limited number of substrates, there is only a single example of an ^{18}O exchange study involving thiol esters in the literature. (This is the study of Bender and Heck already mentioned above[12].) Since this study pertains to the neutral hydrolysis of a thiol ester, there is virtually no information available regarding the

partitioning of the tetrahedral intermediate formed upon hydrolysis of thiol esters in basic media.

Scheme 5:

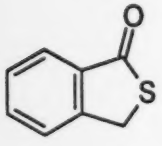


Our interest in the potential reversibility of the anionic tetrahedral intermediates formed during the alkaline hydrolysis of thiol esters was aroused by the observation[20] that a plot of k_{obs} vs. [OH⁻] for thiophthalide (**4**), exhibited a small but clear upward curvaure at high [OH⁻]. This and the fact that **4** hydrolyzes about 10 fold slower than the oxygen analogue **5**, suggested to us that the anionic tetrahedral intermediate produced from **4** might suffer significant reversal concurrent with breakdown to product. Upward curvature in plots of hydrolysis rate vs. [OH⁻] has previously been observed in the hydrolysis of certain amides[21] and has been taken as evidence for a process whereby a second molecule of hydroxide

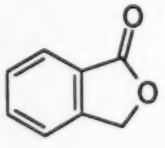
promotes breakdown of the anionic tetrahedral intermediate to products, (k_3 term in **Scheme 5**). The k_3 term will only be kinetically observable when breakdown of the tetrahedral intermediate to product is the rate limiting step (see derivation of rate equations for k_{hyd} and k_{ex} following section).

The only prior account of a simple carboxylic ester hydrolyzing with a second order hydroxide term in its rate law was reported by Khan and Olagbemiro[22], who presented kinetic evidence for the occurrence of an oxydianionic tetrahedral intermediate in the hydrolysis of methyl salicylate and methyl *o*-methoxybenzoate (**7**).

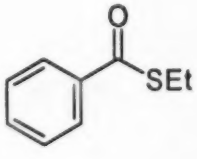
In order to try to demonstrate the existence of a *bona fide* second order in OH^- term in the hydrolysis of a simple carboxylic ester, and to obtain information on the partitioning of the tetrahedral intermediate formed during thiolester hydrolysis in base, we have undertaken a detailed study of the base promoted hydrolysis and ^{18}O exchange kinetics of **4** and **5**, and of ethyl thiolbenzoate (**6**), as well as a reinvestigation of the hydrolysis and exchange of methyl *o*-methoxybenzoate, (**7**).



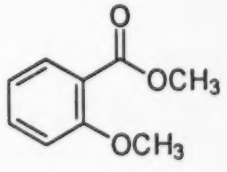
4



5



6



7

Derivations of Equations for k_{ex} and k_{hyd}

(A) Hydrolysis. The most general scheme for the mechanism of hydrolysis of esters in base including a possible pathway for breakdown of To^- through a dianionic tetrahedral intermediate is given in **Scheme 5**. The rate law for such a scheme can be derived as follows:

(6) $d[P]/dt = -d[E]/dt = (k_2 + k_3[OH^-])[To^-]$

where E = ester and P = products (carboxylic acid + alcohol (thiol)).

If it is assumed that To^- is an unstable species, then the steady state approximation can be applied to To^- to obtain the following expression:

(7) $$[To^-] = \frac{k_1[E][OH^-]}{k_{-1} + k_2 + k_3[OH^-]}$$

Substituting this expression for $[To^-]$ into eq. (6) gives:

(8) $$\frac{d[P]}{dt} = \frac{-d[E]}{dt} = \frac{\left(k_1k_2[OH^-] + k_1k_3[OH^-]^2\right)[E]}{k_{-1} + k_2 + k_3[OH^-]} = k_{hyd}[E]$$

so that the observed pseudo first order rate constant for hydrolysis is given by:

(9) $$k_{hyd} = \frac{k_1k_2[OH^-] + k_1k_3[OH^-]^2}{k_{-1} + k_2 + k_3[OH^-]}$$

(Here we are assuming that breakdown of the dianionic tetrahedral intermediate is fast relative to its formation, so that the reverse reaction $To^{-2} \rightarrow To^-$ is neglible. For a mechanism involving reversible formation of To^{-2} with rate limiting breakdown of To^{-2} to product;

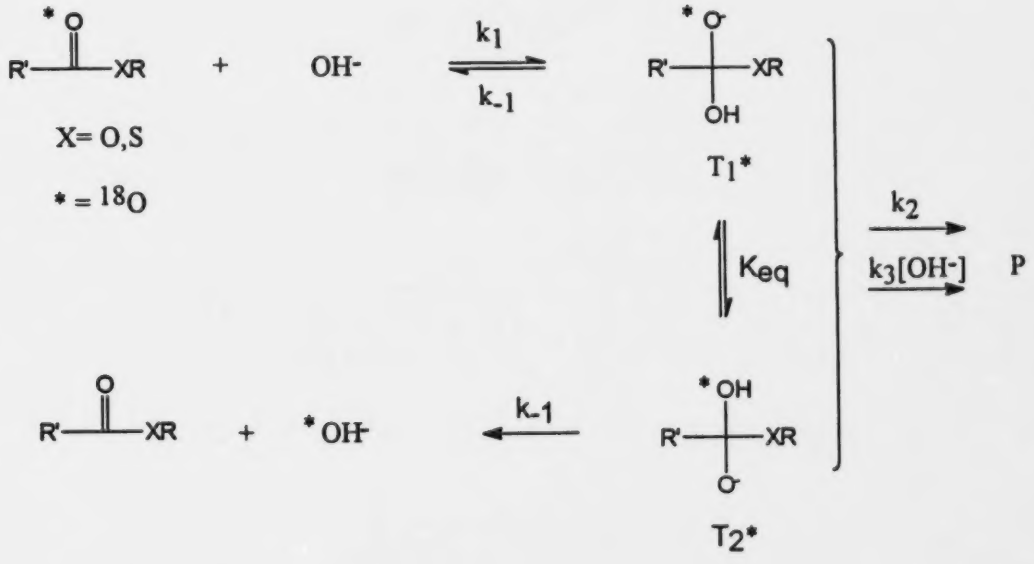
$$To^- \underset{}{\overset{K_{eq}}{\rightleftharpoons}} To^{-2} \xrightarrow{k_4} P$$

the derived rate law will have the same form as eq. **(8)** except that k_3 will be replaced by k_4K_{eq}.)

Equation **(8)** is the most general expression for the rate of ester hydrolysis with no assumptions being made as to which step is rate determining. Note that eq. **(9)** reduces to eq. **(2)** when $k_3 = 0$.

(B) Oxygen-18 Exchange. The process leading to exchange of ^{18}O out of labelled ester is shown in **Scheme 6**:

Scheme 6:



The rate of exchange corresponds to the rate of disappearance of the fraction of ester containing ^{18}O label, equation **(10)**:

$$\textbf{(10)} \qquad \frac{-d\left(\left[E^*\right]/\left[E\right]\right)}{dt} = k_{ex}\left(\frac{\left[E^*\right]}{\left[E\right]}\right)$$

where [E*] = concentration of labelled ester at time t, [E] = concentration of total ester at time t. To obtain an expression for d([E*]/[E])/dt the quotient rule must be used since both [E*] and [E] change as a function of time.

$$\textbf{(11)} \qquad \frac{-d\left(\left[E^*\right]/\left[E\right]\right)}{dt} = \frac{-\left(d\left[E^*\right]/dt\right)}{\left[E\right]} + \left(\frac{\left[E^*\right]}{\left[E\right]^2}\right)\frac{d\left[E\right]}{dt} = k_{ex}\left(\frac{\left[E^*\right]}{\left[E\right]}\right)$$

Separate expressions for d[E*]/dt and d[E]/dt can be obtained for the process in **Scheme 6** by using the steady state approximation for the tetrahedral intermediates, T_1^* and T_2^*.

(i). Derivation of -d[E*]/dt:

$$\textbf{(12)} \qquad \frac{-d\left[E^*\right]}{dt} = k_1\left[E^*\right]\left[OH^-\right] - k_{-1}\left[T_1^*\right]$$

Using the steady state approximation for T_1^* and assuming that the rate of proton transfer between the O's of the tetrahedral intermediate is >> ($k_{-1} + k_2 + k_3[OH^-]$) gives eq. **(13)**.

$$\textbf{(13)} \qquad \frac{d\left[T_1^*\right]}{dt} = 0 = k_1\left[E^*\right]\left[OH^-\right] - \left(k_{-1} + k_2 + k_3\left[OH^-\right]\right)\left[T_1^*\right]$$

$$-K_{eq}\left(k_{-1} + k_2 + k_3\left[OH^-\right]\right)\left[T_1^*\right]$$

Since $K_{eq} = 1$ this gives:

$$\textbf{(14)} \qquad \left[T_1^*\right] = \frac{k_1\left[E^*\right]\left[OH^-\right]}{2\left(k_{-1} + k_2 + k_3\left[OH^-\right]\right)}$$

Substituting **(14)** into **(12)** gives:

$$\textbf{(15)}\qquad \frac{-d[E^*]}{dt} = \frac{\left(k_1k_{-1}[OH^-] + 2k_1k_2[OH^-] + 2k_1k_3[OH^-]^2\right)[E^*]}{2\left(k_{-1} + k_2 + k_3[OH^-]\right)}$$

(ii). Derivation of -d[E]/dt: This is just the rate of hydrolysis of ester and has already been derived above (equation **(8)**):

$$\textbf{(8)}\qquad \frac{-d[E]}{dt} = \frac{\left(k_1k_2[OH^-] + k_1k_3[OH^-]^2\right)[E]}{k_{-1} + k_2 + k_3[OH^-]}$$

Substituting these expressions for -d[E*]/dt and -d[E]/dt into equation **(11)** gives the following expression for the rate of disappearance of fraction of labelled ester:

$$\textbf{(16)}\qquad \frac{-d\left([E^*]/[E]\right)}{dt} = \frac{k_1k_{-1}[OH^-]}{2\left(k_{-1} + k_2 + k_3[OH^-]\right)}\left(\frac{[E^*]}{[E]}\right) = k_{ex}\left(\frac{E^*}{E}\right)$$

Therefore k_{ex}, the pseudo first order rate constant for exchange is given by:

$$\textbf{(17)}\qquad k_{ex} = \frac{k_1k_{-1}[OH^-]}{2\left(k_{-1} + k_2 + k_3[OH^-]\right)}$$

Note that eq. **(17)** reduces to eq. **(1)** when $k_3 = 0$.

(C) Expression for k_{ex}/k_{hyd}. From equation **(9)** and **(17)** the following expression can be derived for k_{ex}/k_{hyd}:

$$\textbf{(18)}\qquad \frac{k_{ex}}{k_{hyd}} = \frac{k_{-1}}{2\left(k_2 + k_3[OH^-]\right)}$$

If $k_3 = 0$ in the above expression (i.e. there is no significant second order in hydroxide term in the hydrolysis rate law), then the following expression for k_{ex}/k_{hyd} obtains:

(19) $$\frac{k_{ex}}{k_{hyd}} = \frac{k_{-1}}{2k_2}$$

Therefore if there is a significant k_3 term the ratio k_{ex}/k_{hyd} will be a function of $[OH^-]$, whereas if $k_3 \approx 0$, k_{ex}/k_{hyd} will be independent of $[OH^-]$.

(D) Limiting Cases for k_{ex} and k_{hyd}.

The following limiting cases can be considered for the exchange and hydrolysis processes.

Case 1: $(k_2 + k_3[OH^-]) >> k_{-1}$. Attack of OH^- is rate limiting for hydrolysis.

In this case $k_{hyd} = k_1[OH^-]$ so that a plot of k_{hyd} vs. $[OH^-]$ will be linear.

The expression for k_{ex} under these circumstances is $k_{ex} = \frac{k_1 k_{-1}[OH^-]}{2(k_2 + k_3[OH^-])}$;

since $\left(k_{-1}/2(k_2 + k_3[OH^-])\right) << 1$, k_{ex} will be $<< k_1[OH^-] = k_{hyd}$. Therefore very little if any ^{18}O exchange will be observed.

Case 2: $k_{-1} \geq (k_2 + k_3[OH^-])$ Breakdown of the tetrahedral intermediate to product is partially or completely rate limiting for hydrolysis. In this case the expression for k_{hyd} is given by eqn. **(8)**: $k_{hyd} = \frac{k_1 k_2[OH^-] + k_1 k_3[OH^-]^2}{k_{-1} + k_2 + k_3[OH^-]}$. (Note that in the case where breakdown to product is completely rate limiting this expression becomes : $k_{hyd} = \frac{k_1 k_2}{k_{-1}}[OH^-] + \frac{k_1 k_3}{k_{-1}}[OH^-]^2$.) In this case a plot of k_{hyd} vs. $[OH^-]$ will exhibit

curvature in certain domains of [OH^-]. Since the tetrahedral intermediate undergoes significant reversal to starting material concurrent with hydrolysis under these conditions, a large amount of ^{18}O exchange should be observable. (The expression for k_{ex} is given by eq. **(16)** in this case.)

**Note: In the case where breakdown to product is partially or completely rate limiting but no pathway leading to the formation of a dianionic tetrahedral intermediate exists (i.e. $k_3 \approx 0$), the expression for k_{hyd} becomes:

$k_{hyd} = \frac{k_1 k_2 [OH^-]}{k_{-1} + k_2}$, and a plot of k_{hyd} vs. [OH^-] will be linear. Under these circumstances rate limiting breakdown of the tetrahedral intermediate becomes kinetically indistinguishable from rate limiting attack of [OH^-]. Therefore in the absence of a significant k_3 term the identity of the rate limiting step can only be determined by carrying out ^{18}O exchange experiments.

Experimental

(A) Materials and General Methods.

The following compounds were obtained from commercial suppliers: phthalide (Aldrich), *o*-anisic acid (or *o*-methoxybenzoic acid, Sigma), benzoyl chloride (Fluka), ethyl benzoate (Terrochem).

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker WH-200 or a Bruker AM-400 spectrometer. Infrared spectra were recorded on a Nicolet 7199 or a Nicolet Magna 750 FTIR spectrometer. High resolution mass spectra were obtained on an AEI-MS50 mass spectrometer and low resolution spectra on an AEI-MS12 spectrometer.

All melting points were obtained on a Canlab Gallenkamp apparatus and are uncorrected.

Flash chromatography was performed using silica gel 60 (40-63 μm particle size).

(B) Syntheses.

2-(Benzylmercaptomethyl)benzoic Acid (8). This compound was obtained from phthalide and benzylthiol via the method described by Lumma et al.[23] The crude orange solid was recrystallized once from $MeOH/H_2O$ and twice from ether/petroleum ether to give a pink solid in 58% yield. This material was used without further purification: mp 106-110°C, lit.[24] 127°C; IR ($CHCl_3$ cast film) ν 3200-2500 (OH), 1683 (C=O), 1574, 1400, 1299, 1268 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 2 H), 4.10 (s, 2 H), 7.20-

7.38 (m, 7 H), 7.48 (t, J = 8.0 Hz, 1 H), 8.07 (d, J = 8.0 Hz, 1 H); HRMS, exact mass calc'd for $C_{15}H_{14}O_2S_1$ 258.0714, found 258.0718.

2-Thiophthalide (4). To 75 mL of dry benzene was added 2.5 g (0.0097 mol) of the acid **8** (mixture is cloudy). A few drops of pyridine were added and the temperature lowered to 0°C (under Ar). Oxalyl chloride, 0.7 mL (0.08 mol), was then added via syringe. The mixture became a clear orange but after several hours of stirring it became cloudy again. The next day benzene and excess oxalyl chloride were removed by distillation. The brown residue was chromatographed on a silica gel flash column, 80% hexane/20% EtOAc, and then recrystallized from ether/hexane to give 0.6 g of **4** (white solid, 41% yield): mp 56-58°C, lit.[25], 56-58°C; IR ($CHCl_3$ cast film) ν 1674 (C=O), 1604, 1585, 1486, 1241, 906 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.48 (s, 2 H), 7.48 (t, 1 H, J_{app} = 8 Hz), 7.54 (d, 1 H, J_{app} = 8 Hz), 7.63 (t, 1 H, J_{app} = 8 Hz), 7.84 (d, 1 H, J_{app} = 8 Hz).

^{18}O-Labelled 2-(Mercaptomethyl)benzoic Acid (9). To a 100 mL flask containing 60 mL of dry MeOH was added 0.97 g (0.0064 mol) of dry thiophthalide **4**. The solution was cooled to 0°C, under Ar, and then 0.294 g (0.0128 mol) of Na was carefully added. When H_2 evolution had ceased, 0.231 mL (0.0128 mol) of 98% $H_2{}^{18}O$ was added to the solution. The mixture was heated at reflux for 14 days. The MeOH was then removed and the solid residue dissolved in 20 mL of H_2O; then conc. HCl was added dropwise until no further precipitation was observed (pH = 2). The solid was rapidly filtered and then washed with about 5 mL of cold H_2O. After drying, 0.6 g of a crude

pink solid was obtained (56% yield): IR ($CHCl_3$ cast film) ν 3200-1800 (OH), 1669 (C=O), 1572, 1403, 1298, 1273 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.13 (t, 1 H, SH), 4.12 (s, 2 H, CH_2-S-S-CH_2), 4.12 (d, 2 H, CH_2-SH), 7.25-8.15 (m, 4 H); HRMS, exact mass calc'd for $C_8H_8{}^{16}O{}^{18}OS_1$ 170.0288, found 170.0288 (52%). (Also observed was a peak at 172.0319 (14%) due to $C_8H_8{}^{18}O_2S_1$).

From the NMR spectrum there appeared to be about 20% of disulfide present. However this mixture was used directly to make the labelled thiolester without attempting to purify the thiol acid from the disulfide.

^{18}O-Labelled 2-Thiophthalide (4-^{18}O). To 60 mL of dry benzene was added 0.6 g (0.0035 mol) of the crude labelled thiol acid **9** (mixture not completely soluble), followed by 1.2 equivalents of $SOCl_2$. The mixture was stirred for 0.5 h at room temperature, and then heated at reflux overnight after adding an extra 2 equivalents of $SOCl_2$ (residue dissolves as reaction proceeds). Benzene and excess $SOCl_2$ were removed by distillation. The crude material was purified as described for **4**. TLC and GC indicated a single component was present. Yield of **4**-^{18}O, 0.17 g (32%) of a white solid: IR ($CHCl_3$ cast film) ν 1676 (C=^{16}O), 1642 (C=^{18}O) cm^{-1}; ^1H NMR ($CDCl_3$) identical to that for compound **4**; ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.63, 123.9, 126.3, 127.9, 133.1, 135.9, 147.0, 197.71 (C=^{18}O), 197.75 (C=^{16}O); HRMS, exact mass calc'd for $C_8H_6{}^{16}O_1S_1$ 150.0139, found 150.0142 (81%), exact mass calc'd for $C_8H_6{}^{18}O_1S_1$ 152.0183, found 152.0178 (88%).

^{18}O-Labelled 2-Hydroxymethylbenzoic Acid (10). This compound was synthesized from phthalide in an identical manner to the labelled thiol acid **9**, except that the reaction was over after 48 h. Compound **10** was obtained as a white solid in 82% yield (crude): mp 110-112°C, lit.[26] 127-128°C; IR (MeOH cast film) ν 1669 (C=^{16}O), 1657 (C=^{18}O) cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 4.94 (s, 2 H), 5.38 (s, phthalide, -C*H*$_2$-), 7.35 (t, 1 H, J_{app} = 8 Hz), 7.55 (t, 1 H, J_{app} = 8 Hz), 7.64 (d, 1 H, J_{app} = 8 Hz), 7.97 (d, 1 H, J_{app} = 8 Hz). Small peaks due to phthalide were present in the aromatic region. Their intensity is ~ 10% of the peaks attributable to **10.** It is believed that this was formed by cyclization of the labelled hydroxy acid during the acidic workup rather than being due to unreacted starting material. HRMS, excact mass calc'd for $C_8H_8{}^{16}O_2{}^{18}O$ 154.0516, found 154.0516 (33%). A peak was also observed at 156.0559 (6%), corresponding to $C_8H_8{}^{16}O_1{}^{18}O_2$.

^{18}O-Labelled Phthalide (5-^{18}O). To 75 mL of dry $CHCl_3$ was added 0.7 g (0.0045 mol) of the hydroxy acid **10**. $SOCl_2$ (1.5 eq.) was added and the mixture was stirred at room temperature overnight (condenser, drying tube). Solvent and excess $SOCl_2$ were removed and the residue purified by flash chromatography (70% petroleum ether/30% EtOAc, silica), giving (5-^{18}O) in quantitative yield. One peak was observed by TLC and GC. Mixture mp (authentic phthalide + 5-^{18}O), 72-74°C, lit.[27] 72-74°C; IR ($CHCl_3$ cast film) ν 1757 (C=^{16}O), 1727 (C=^{18}O), 1466, 1439, 1054, 1017 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28 (s, 2 H), 7.48 (m, 2 H), 7.65 (t, 1 H, J_{app} = 7.5 Hz),

7.86 (d, 1 H, J_{app} = 7.5 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 71.38, 123.65, 126.17, 126.52, 130.07, 135.41, 148.74, 173.33 ($C=^{18}O$), 173.37 ($C=^{16}O$); HRMS, exact mass calc'd for $C_8H_6{}^{16}O_1{}^{18}O_1$ 136.0411, found 136.0410 (52%), exact mass calc'd for $C_8H_6{}^{16}O_2$ 134.0368, found 134.0366 (43%).

***o*-Methoxybenzoyl Chloride (11)**. The acid chloride of *o*-methoxybenzoic acid was made in the standard way using $SOCl_2$ as solvent. The crude acid chloride was purified by Kugelrohr distillation, giving **11** as a clear liquid in quantitative yield: IR (neat film) ν1782 (C=O), 1733, 1600, 1482, 1286, 861 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.92 (s, 3 H), 7.0 (t, 2 H), 7.55 (t, 1 H), 8.05 (d, 1 H).

Methyl *o*-Methoxybenzoate (7). Dry pyridine (15 mL) was added to 0.5 g (0.0029 mol) of the acid chloride **11**. A white precipitate formed immediately. Then 250 μL (0.0058 mol) of dry MeOH was slowly added. After 2 h the reaction mixture was filtered, and the filtrate subjected to rotary evaporation to remove excess pyridine and MeOH. The residue was dissolved in 30 mL of CH_2Cl_2 and extracted with 3 x 10 mL of 0.5 M HCl. The $CHCl_3$ layer was dried over Na_2CO_3, filtered, and the $CHCl_3$ removed. The crude yellow liquid was purified by distillation (bp 70-80°C, 0.1 Torr., lit.[28] 127-127.5°C, 11 Torr.) to give **7** in 81% yield (from the acid): IR ($CHCl_3$ cast film) ν 1729 (C=O), 1601, 1492, 1436, 1304, 1253, 1085 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.92 (s, 3 H), 3.93 (s, 3 H), 6.93-7.02 (m, 2 H), 7.43-7.52 (m, 1 H), 7.80 (dd, 1 H, J_{app} = 7.5, 2 Hz); HRMS, exact mass calcd. for $C_9H_{10}O_3$ 166.0630, found 166.0630 (35%).

^{18}O-Labelled Methyl *o*-Methoxybenzoate (7-^{18}O). The labelled compound was made by the following series of steps. First the acid chloride, **11**, was hydrolyzed in 98% $H_2{}^{18}O$, to give the labelled acid. This was in turn converted via $SOCl_2$ into the 50% ^{18}O labelled acid chloride (**11**-^{18}O) which was then converted to the labelled ester (**7**-^{18}O) by the same procedure as described above for **7**. IR (neat film) ν 1730 (C=^{16}O), 1701 (C=^{18}O) cm^{-1}; ^{1}H NMR identical to that for **7**; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 51.92, 55.94, 112.01, 120.05, 120.09, 131.59, 133.44, 159.08, 166.63 (C=^{18}O), 166.67 (C=^{16}O); HRMS, exact mass calc'd for $C_9H_{10}{}^{16}O_3$ 166.0630, found 166.0630 (9.3%), exact mass calc'd for $C_9H_{10}{}^{16}O_2{}^{18}O_1$ 168.0673, found 168.0672.

Ethyl Thiobenzoate (6). To a 25 mL flask under Ar was added 10.4 mL (0.140 mol) of EtSH and 2.3 mL (0.028 mol) of dried pyridine. Benzoyl chloride (1.65 mL, 0.014 mol) was then added via syringe with cooling. A white precipitate formed immediately. After 24 h, 20 mL of cold ether was added to the mixture and the pyridinium hydrochloride was removed by filtration. The ether phase was then extracted with 2 x 10 mL of 1.4 M HCl and then with 10 mL of 10% K_2CO_3, dried over $MgSO_4$, filtered, and the ether removed *in vacuo* to give a clear liquid. The thiol ester was purified by Kugelrohr distillation (bp 70-80°C, 0.075 Torr., lit.[29] 146°C, 31 Torr.), and then by flash chromatography on silica gel (90% hexane/10% EtOAc) to yield 2.2 g of material (95% yield); IR (neat film) ν 1662 (C=O), 1208, 912, 690 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.37 (t, 3 H), 3.08 (q, 2 H), 7.40-7.50 (m, 2 H), 7.53-7.62 (m, 1 H), 7.95-8.02

(m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.78, 23.45, 127.18, 128.57, 133.23, 137.28, 192.08; HRMS, exact mass calcd. for $C_9H_{10}O_1S_1$, 166.0452, found 166.0447 (14%).

^{18}O-Labelled Ethyl Thiobenzoate (6-^{18}O). The ^{18}O labelled ester was made from benzoyl chloride in a series of steps identical to that described for compound 7-^{18}O, with the exception that the final step (conversion of the labelled acid chloride to the thiol ester), was carried out as described for **6**. IR (neat film) ν 1662 (C=^{16}O), 1632 (C=^{18}O) cm^{-1}; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.07 (C=^{18}O), 192.11 (C=^{16}O); HRMS, exact mass calc'd for $C_9H_{10}{}^{16}O_1S_1$ 166.0453, found 166.0452 (15.5%), exact mass calc'd for $C_9H_{10}{}^{18}O_1S_1$ 168.0496, found 168.0495 (12.82%). (Other spectral characteristics identical to those for **6**.)

(C) Kinetics.

Hydrolysis. All base solutions were made by dilution of 19 M NaOH in a dry box (CO_2 free conditions) using degassed (CO_2 free, O_2 free), deionized water (Osmonics-Aries water purifying system). KCl was used to maintain constant ionic strength. NaOH solutions were titrated with standard HCl solution (Aldrich), using bromothymol blue as indicator, or with potassium hydrogen phthalate using phenolphthalein as indicator.

(i) Thiophthalide (T = 25°C, μ = 3.0 (KCl)). Rate constants for the basic hydrolysis of thiophthalide were obtained under pseudo-first order conditions by following the decrease in absorbance at 270 nm of thiophthalide, using a modified Cary 17 spectrophotometer interfaced with an IBM 486 microcomputer using OLIS software

(Online Instrument Systems, Jefferson, GA., 1992). In all cases 3 mL of base solution was added to the cuvette in the dry box and then Ar was bubbled through the solution in the cell to remove O_2 (less than 1 minute). The stoppered cuvette was allowed to equilibrate in the spectrophotometer cell holder for about 10 min., and then 8 uL of a 0.03 M solution of thiol ester in DME was injected into the cell to initiate the run ($[ester]_{uv\ cell} = 8x10^{-5}$ M). Reactions were followed to at least 5 half lives, and runs were performed in triplicate for each base concentration. A span of NaOH concentrations ranging from 0.01-3.0 M was examined.

(ii) Phthalide (T = 25°C, μ = 3.0 (KCl)). For NaOH concentrations of 0.0108 M and 0.108 M, the modified Cary 17 spectrophotometer was used to follow the kinetics, using the same procedure as for thiophthalide (except that the final concentration of phthalide in the UV cell was 4.0×10^{-4} M) and the wavelength used was 281 nm. For NaOH concentrations of 0.725-2.06 M, stopped flow kinetics were employed using a Durrum-Gibson D-110 Stopped Flow Spectrophotometer. A Cantech Scientific transient recorder was interfaced with an IBM PS2 microcomputer and first order rate constants were extracted from the absorbance vs. time data via the program 'Exponential Kinetic Fit' (Version 1.2 Cantech Scientific, 1988). One drive syringe of the instrument was filled with NaOH solution (twice the desired final concentration) and the other drive syringe filled with 8.0×10^{-4} M phthalide (4% DME), with the ionic strengths of the two solutions adjusted with KCl so that the final ionic strength in the mixing cell would be 3 M. Once the drive syringes were filled with their respective solutions, they were allowed

to equilibrate in the water bath for 15-20 min. About 10 runs were performed at each base concentration and the average of the k_{obs} values taken.

(iii) Hydrolysis of Phthalide and Thiophthalide in D_2O/NaOD (T = 25°C, μ = 3.0 (KCl)). Thiophthalide: solutions ranging in concentration from ~ 0.01 M - 2.2 M NaOD were made up by adding Na to an ice cooled solution of D_2O under Ar. The determination of the base concentrations and hydrolysis kinetics were carried out as described above.

Phthalide: hydrolysis of phthalide in D_2O was carried out in an identical manner but only using two NaOD concentrations (0.0097 M and 0.0941 M).

(iv) Methyl *o*-Methoxybenzoate (T = 35°C, μ = 2.0 (KCl)). The hydrolysis of methyl *o*-methoxybenzoate was followed by observing the decrease in absorbance at 310 nm using the same procedure as described for thiophthalide hydrolysis. A span of NaOH concentrations ranging from 0.005-1.0 M was examined.

(v) Ethyl Benzoate and Ethyl Thiobenzoate (T = 25°C, μ = 2.0 (KCl)). The hydrolysis kinetics were followed by UV spectroscopy as described for thiophthalide except for the following changes: assay wavelength for ethyl benzoate = 245 nm, $[ester]_{uv\ cell} = 4.2 \times 10^{-4}$ M; assay wavelength for ethyl thiobenzoate = 275 nm, $[ester]_{uv\ cell} = 1.7 \times 10^{-4}$ M. The esters were hydrolyzed in NaOH solutions ranging in concentration from ~ 0.001-2.0 M.

^{18}O-Exchange Studies. General procedure for ^{18}O-exchange experiments: 100 mL of NaOH solution was added to a 100 mL volumetric flask in an Ar filled dry box

(CO_2 free conditions). The septum-capped flask was then equilibrated at the required temperature for ~ 15 min. A small aliquot of a concentrated solution of the ester in DME or EtOH was injected into the flask, to give a final concentration of ester on the order of 4 x 10^{-4} M. The flask was inverted several times and the reaction mixture left in the water bath for the requisite amount of time (up to 3 half times of hydrolysis). The reaction mixture was quenched by quickly pouring it into 10 mL of 1.1 M phosphate buffer, pH 6.5. In cases where hydrolysis was very slow, the reaction solution was simply extracted quickly without quenching. The aqueous solution was extracted with 3 x 30 mL of distilled CH_2Cl_2. The combined CH_2Cl_2 extracts were then dried, filtered, and the volatiles removed. The residue was dissolved in 10 drops of CH_2Cl_2 and transferred to a vial for low resolution mass spectral analysis.

The % ^{18}O in the recovered ester for each sample was then determined by taking the average over 22 scans of the parent peaks and applying the equation % $^{18}O = 100 \times I_{M+2} / (I_M + I_{M+2})$ where I_{M+2} = the intensity of the M+2 peak corresponding to the ^{18}O labelled ester, and I_M = the intensity of the mass M peak corresponding to the unlabelled ester.

Prior to the actual exchange experiments, a control experiment was performed to show that ^{18}O label is not lost from ester left to sit in pH 6.5 phosphate buffer (quenching buffer). The labelled ester (5 mg) was added to 100 mL of 0.1 M phosphate buffer and left for 2 h stirring. The ester was then extracted from the aqueous solution with CH_2Cl_2 as described above and submitted to mass spectral analysis. No ^{18}O label was lost from the ester.

Results

(A) Hydrolysis.

Given in **Table 1** are the pseudo-first order rate constants (k_{hyd}) for the hydrolysis of thiophthalide **(4)** and phthalide **(5)** at various base concentrations in both H_2O and D_2O (T = 25°C, μ = 3.0 (KCl)). The data for a given ester cannot be satisfactorily fit by a linear regression of the type:

(20) $$k_{hyd} = k_O + B\,[OL^-]$$

(where L = H or D) but can be fit by an expression that includes both first and second order in $[OL^-]$ components, e.g.,

(21) $$k_{hyd} = k_O + B\,[OL^-] + C\,[OL^-]^2$$

Given in **Fig. 1** are graphical presentations of the data. The lines through the data are those computed on the basis of fits of the k_{hyd} vs. $[OL^-]$ values to eq. **(21)** and clearly show an upward deviation from a linear regression. This deviation is also seen under conditions of μ = 1.0 (KCl) up to 1 M $[OH^-]$, but is more clearly defined under the conditions used here. The hydrolysis data for the open analogues of the phthalides, namely ethyl benzoate and ethyl thiobenzoate **(6)** (T = 25°C, μ = 2.0 (KCl)), are given in **Table 2**. Each of these esters also shows some slight upward curvature in the plots of k_{hyd} vs. $[OH^-]$ (**Fig. 2**), and the data can be fit by eq. **(21)**. Given in **Table 3** are the best fit parameters for these esters. The ratio of B/C (M) given in column 4 is indicative of the relative ratio of the computed first and second order in $[OH^-]$ terms.

The hydrolysis of methyl *o*-methoxybenzoate was also carried out under the same conditions reported by Khan and Olagbemiro[22] (T = 35°C, μ = 2.0 (KCl)).(See **Table 2** for measured rate constants). The rate constants B and C obtained from NLLSQ fitting of the data to eq. **(21)**, along with the corresponding rate constants obtained by Khan, are given in **Table 3**. There is obviously a large discrepancy betwen our results and those reported by Khan under reportedly identical conditions. According to Khan, the $C[OH^-]^2$ term contributes 50% to k_{hyd} at 0.37 M NaOH while our results indicate that this term is negligible and will contribute 50% to k_{hyd} only at 9.5 M NaOH. In fact our data is fit equally well by linear regression ($k_{hyd} = k_2[OH^-]$, where $k_2 = 0.0723 \pm 0.0004$ $M^{-1}s^{-1}$) as shown in **Fig. 4** (solid line). Included in this figure is the theoretical curve calculated from Khan's results (dashed line).

Table 1. Pseudo-First Order Hydrolysis Rate Constants for Thiophthalide and Phthalide in Basic Media, T = 25°C, μ = 3.0 (KCl).

Ester		[NaOL] (M)	$k_{hyd}(s^{-1})^a$
Thiophthalide (4)	D_2O	0.0083	$(2.46\pm0.04) \times 10^{-4}$
		0.0106	$(3.23\pm0.02) \times 10^{-4}$
		0.108	$(2.47\pm0.01) \times 10^{-3}$
		0.956	$(2.85\pm0.04) \times 10^{-2}$
	D_2O	0.967	$(3.46\pm0.02) \times 10^{-2}$
		1.45	$(4.49\pm0.01) \times 10^{-2}$
	D_2O	1.89	$(7.66\pm0.09) \times 10^{-2}$
		1.92	$(6.58\pm0.06) \times 10^{-2}$
	D_2O	2.22	0.094±0.002
		3.01	0.119±0.005
Phthalide (5)	D_2O	0.0097	$(3.07\pm0.07) \times 10^{-3}$
		0.0108	$(2.66\pm0.01) \times 10^{-3}$
	D_2O	0.0941	$(3.27\pm0.02) \times 10^{-2}$
		0.108	$(2.88\pm0.02) \times 10^{-2}$
		0.725	0.201±0.004
		0.960	0.280±0.008
		1.50	0.482±0.003
		1.83	0.625±0.014
		2.06	0.747±0.025

[a] Error in k_{hyd} is given as ±1 standard deviation in the mean calculated from 3 replicate kinetic runs.

Figure 1. Plots of k_{hyd} vs. [OL^-] data for hydrolysis of thiophthalide **(4)** and phthalide **(5)** in H_2O and D_2O media, T = 25°C, μ = 3.0 (KCl). (thiophthalide ● (H_2O); O (D_2O); phthalide ▲ (H_2O); lines through the data computed on the basis of NLLSQ fitting to eq. **(21)**).

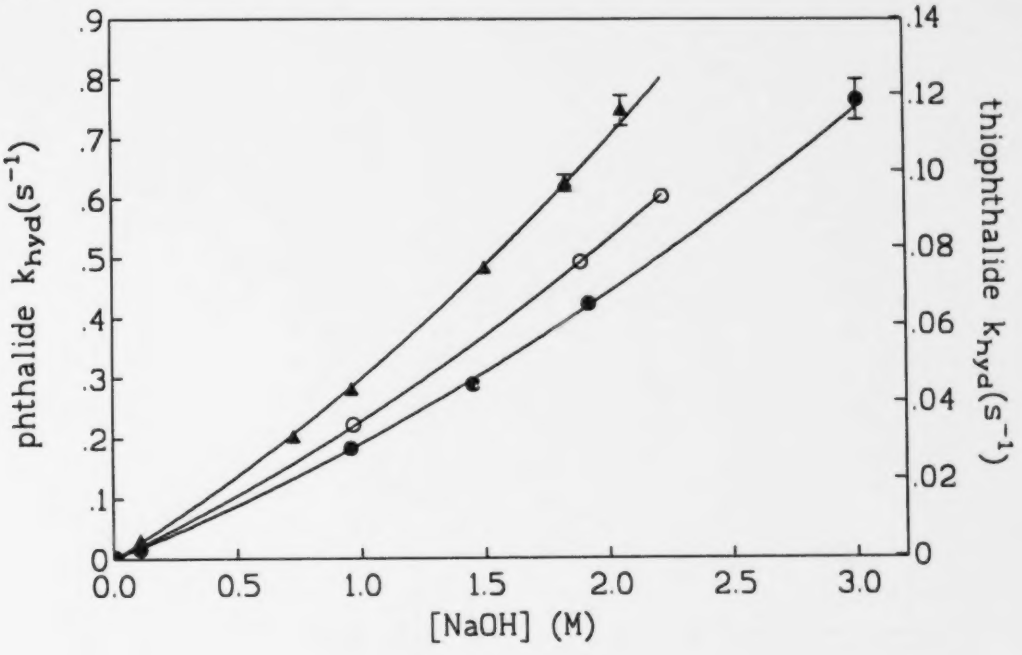


Table 2. Pseudo-First Order Hydrolysis Rate Constants for Ethyl Thiobenzoate and Ethyl Benzoate, T = 25°C, μ = 2.0 (KCl), and Methyl *o*-Methoxybenzoate, T = 35°C, μ = 2.0 (KCl), in Basic Media.

Ester	[NaOH] (M)	$k_{hyd}(s^{-1})$[a]
Ethyl thiobenzoate (**6**)	0.00099	$(2.62\pm0.09) \times 10^{-5}$
	0.0049	$(1.47\pm0.04) \times 10^{-4}$
	0.010	$(3.13\pm0.06) \times 10^{-4}$
	0.050	$(1.56\pm0.02) \times 10^{-3}$
	0.527	$(1.81\pm0.04) \times 10^{-2}$
	0.951	$(3.43\pm0.08) \times 10^{-2}$
	2.12	$(8.7\pm0.2) \times 10^{-2}$
Ethyl benzoate	0.00518	$(7.7\pm0.2) \times 10^{-5}$
	0.00982	$(2.89\pm0.08) \times 10^{-4}$
	0.049	$(1.58\pm0.005) \times 10^{-3}$
	0.527	$(1.95\pm0.004) \times 10^{-2}$
	0.936	$(3.61\pm0.03) \times 10^{-2}$
	2.12	$(9.44\pm0.07) \times 10^{-2}$
Methyl *o*-methoxybenzoate (**7**)	0.00518	$(3.30\pm0.03) \times 10^{-4}$
	0.00982	$(6.52\pm0.02) \times 10^{-4}$
	0.0491	$(3.33\pm0.01) \times 10^{-3}$
	0.0749	$(4.9\pm0.1) \times 10^{-3}$
	0.0992	$(6.83\pm0.01) \times 10^{-3}$
	0.1963	$(1.35\pm0.02) \times 10^{-2}$
	0.963	0.068±0.004

[a] Error in k_{hyd} is given as ±1 standard deviation in the mean calculated from 3 replicate kinetic runs.

Figure 2. Plots of k_{hyd} vs. [OH^-] for hydrolysis of ethyl benzoate (■) and ethyl thiobenzoate (*) in H_2O, T = 25°C, μ = 2.0 (KCl). Lines through the data are computed on the basis of NLLSQ fitting to eq. **(21)**.

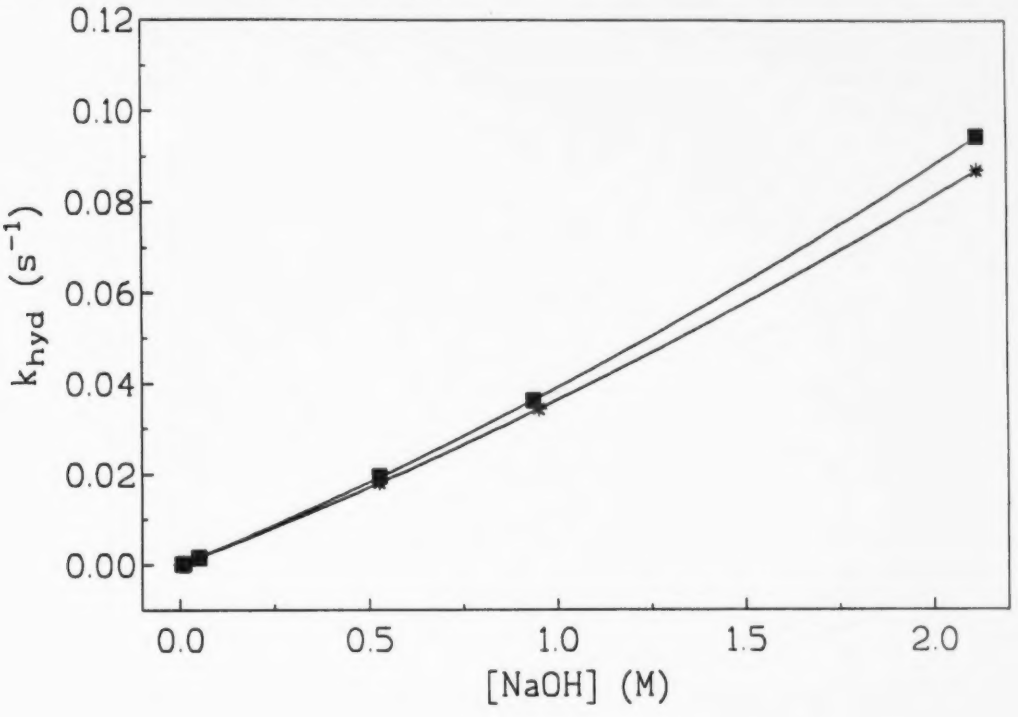


Table 3. Parameters Determined from Nonlinear Regression Fitting of k_{hyd} vs. [NaOH] Data to Eq. 2.[a,b]

Ester	B ($M^{-1}s^{-1}$)	C ($M^{-2}s^{-1}$)	B/C (M)[g]
Thiophthalide[c] (H_2O)	0.025±0.002	0.004±0.001	9.0-4.6
Thiophthalide[c] (D_2O)	0.030±0.001	0.006±0.001	7.75-4.14
Phthalide[c]	0.249±0.005	0.050±0.004	5.52-4.52
Methyl *o*-Methoxybenzoate[d] (this study)	0.066±0.001	0.007±0.002	13.4-7.22
Methyl *o*-Methoxybenzoate[e]	0.031±0.001	0.083±0.008	0.43-0.33
Ethyl Thiobenzoate[f]	0.0321±0.0001	0.0042±0.0001	7.85-7.44
Ethyl Benzoate[f]	0.0341±0.0002	0.0049±0.0001	7.14-6.78

[a] Fitting was carried out using 'Inplot Version 4.0' (Graphpad Software, San Diego, 1992).

[b] k_o in eq. **(21)** (1st order rate constant for background H_2O reaction) was assigned a constant value of zero prior to fitting since the program calculated a negative value for this parameter when it was allowed to vary.

[c] T = 25°C, μ = 3 (KCl)

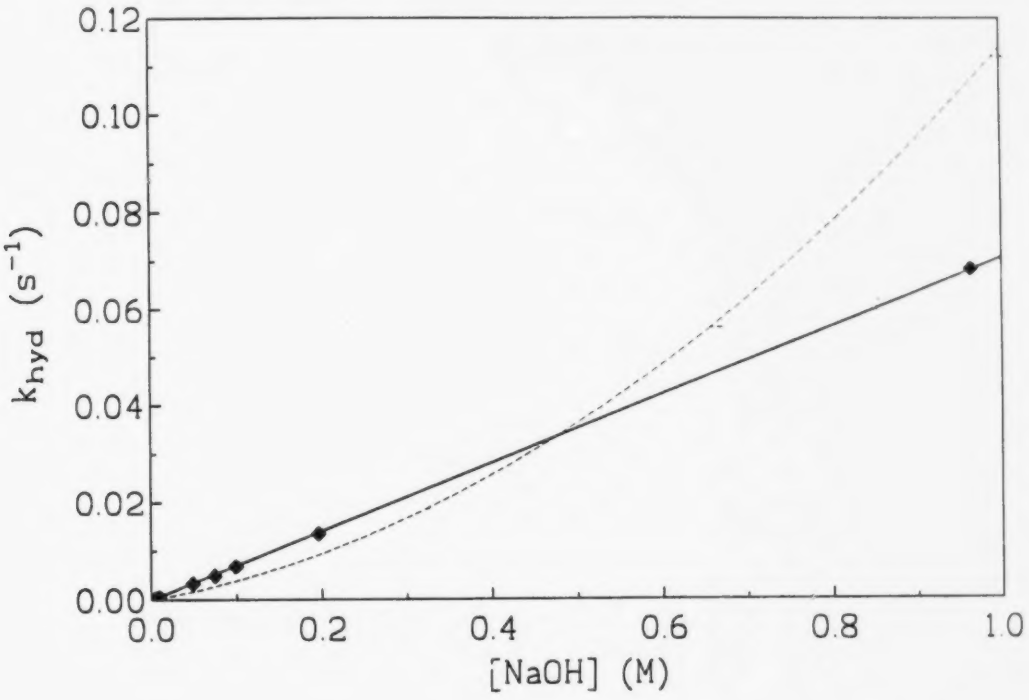
[d] T = 35°C, μ = 2 (KCl)

[e] T = 35°C, μ = 2 (KCl) data of Khan and Olagbemiro, ref. 22.

[f] T = 25°C, μ = 2 (KCl)

[g] Range computed from the standard deviations in B and C.

Figure 3. Plot of the k_{hyd} vs. [OH^-] data for hydrolysis of methyl *o*-methoxybenzoate in H_2O, T = 35°C, μ = 2.0 (KCl). (■, experimental points obtained in this study; solid line computed from linear regression fitting of the data to eq. **(20)**; dashed line, theoretical curve computed on the basis of results of Khan and Olagbemiro.)



(B) ^{18}O-Exchange.

Roughly 50% ^{18}O labelled ester (**4, 5, 6 or 7**) was subjected to the hydrolysis conditions for various times up to 1.5 -2 $t_{1/2}$ for hydrolysis, and then recovered from the reaction medium. The ^{18}O-content was determined from ~ 20 scans of the M^+ and M^++2 peaks (low resolution), and compared with the ^{18}O-content of the unhydrolyzed ester. Under no circumstances did we observe any differences, outside experimental error, for the ^{18}O content relative to the time zero samples. (For original data, see **Tables A-1** to **A-4**, Appendix 1-1).

Discussion

(A) Hydrolysis and ^{18}O Exchange of Phthalide and Thiophthalide.

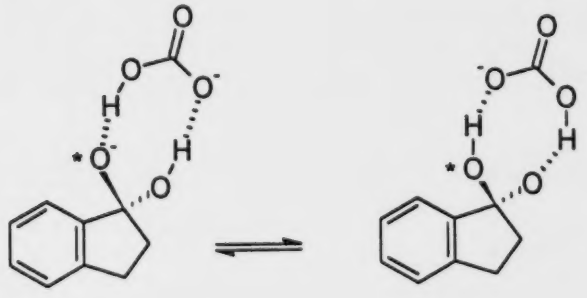
Upward curvature in k_{hyd} vs. [OH^-] profiles for carboxylic acid derivatives has previously been interpreted[21] in terms of a route for breakdown of the tetrahedral intermediate (To^-) to products whereby a second OH^- removes a proton from To^- to form a dianionic tetrahedral species which rapidly decomposes to products. As already pointed out in the introduction, the ability to observe such a second order term kinetically requires that breakdown of the tetrahedral intermediate is the rate limiting step. If this is so, then attack of OH^- on the ester carbonyl should be readily reversible and ^{18}O exchange out of labelled ester should be observable. The validity of such a mechanism involving passage through a dianionic tetrahedral intermediate has been demonstrated for the hydrolysis of a number of amides[9] where both upward curvature in the k_{hyd} vs. [OH^-] plot and ^{18}O exchange out of labelled amide have been observed.

Based on these prior observations we expected phthalide and thiophthalide to exhibit ^{18}O exchange concurrent with hydrolysis since significant curvature was observed in the k_{hyd} vs.[OH^-] plots for both of these esters. However when exchange experiments were carried out with ~ 50% ^{18}O labelled **4** and **5**, no loss of label was observed in either case after up to 2 half times of hydrolysis[30]. There are several explanations that can be offered to account for the presence of curvature in the k_{hyd} vs. [OH^-] plot and the coincident lack of ^{18}O exchange observed for these esters. These are detailed below.

1). Breakdown of To^- to product is rate limiting but no ^{18}O exchange is observed because the oxygens in the tetrahedral intermediate are not equilibrated. In this case

reversal of the tetrahedral intermediate to starting material is not accompanied by exchange because proton transfer between the oxygens of To$^-$ required for expulsion of $^{18}OH^-$ is slow relative to breakdown ($k_{eq} << k_{-1} + k_2 + k_3[OH^-]$, **Scheme 6**). The possibility that the O's of the tetrahedral intermediate are not protonically equilibrated has been suggested by Bender and Thomas[5c] to account for the variation in k_{ex}/k_{hyd} observed in the hydrolysis of a number of *p*-substituted methyl benzoates. In that paper it was suggested that incomplete oxygen equilibration might be demonstrable for ^{18}O exchanging esters by examining the solvent kinetic isotope effect on k_{ex}/k_{hyd}, since if proton transfer were limiting the exchange process, the k_{ex}/k_{hyd} ratio should be greater in H_2O than in D_2O. This type of study has been carried out for the neutral hydrolysis of ethyl trifluoroacetate[12] and the base catalyzed hydrolysis of various amides[9,21h] however this method is only possible for systems that exhibit some ^{18}O exchange. The approach taken by us was to attempt to facilitate the proton transfer between the 2 oxygens of To$^-$ by adding bicarbonate to the medium. It was hoped that bicarbonate would act as a bifunctional catalyst to transfer a H^+ between the 2 oxygens in a single concerted process as shown in equation **(22)**.

(22)

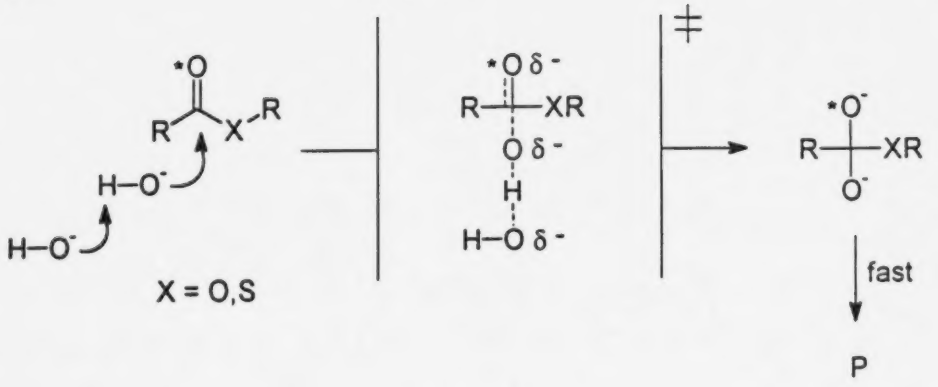


This sort of bifunctional catalysis has previously been demonstrated for bicarbonate and dihydrogen phosphate acting as general acid/base catalysts in the breakdown of both neutral and anionic tetrahedral intermediates[31].

When ^{18}O exchange experiments were carried out in bicarbonate buffer (pH 9.81, phthalide; pH 9.56, thiophthalide; $[Buffer]_T$ = 0.800M) no ^{18}O exchange was observable after up to 2 half times of hydrolysis. This could mean that either HCO_3^- is not able to catalyze oxygen equilibration to a great enough extent so that exchange is observable or that in fact no reversal of the tetrahedral intermediate to starting material is occurring. If the latter is true, which seems most probable, then attack of OH^- must be rate determining and the apparent curvature in the plot of k_{obs} vs. $[OH^-]$ cannot be attributed to a pathway for breakdown of To^- via the dianionic tetrahedral intermediate To^{-2} as shown in **Scheme 5**.

2). A second possibility is that the observed upward curvature but lack of ^{18}O exchange is due to the coexistence of two independent hydrolysis pathways for these esters, one being the conventional hydrolysis route with rate limiting attack of OH^- and the second involving two molecules of OH^- in the rate limiting transition state as shown in **Scheme 7**. A combination of these two paths would lead to the observed rate law, equation **(21)**. Because the process depicted above involves a proton in flight in the rate limiting transition state, a large normal solvent kinetic isotope effect (SKIE) would be expected for the rate constant for this process (C, eq. **(21)**). However when the hydrolysis of thiophthalide was carried out in D_2O, inverse isotope effects ($k^{H_2O}/k^{D_2O} < 1$) were found on both B and C ($B^{H_2O}/B^{D_2O} = 0.83 \pm 0.09$, $C^{H_2O}/C^{D_2O} = 0.67 \pm 0.24$).

Scheme 7:

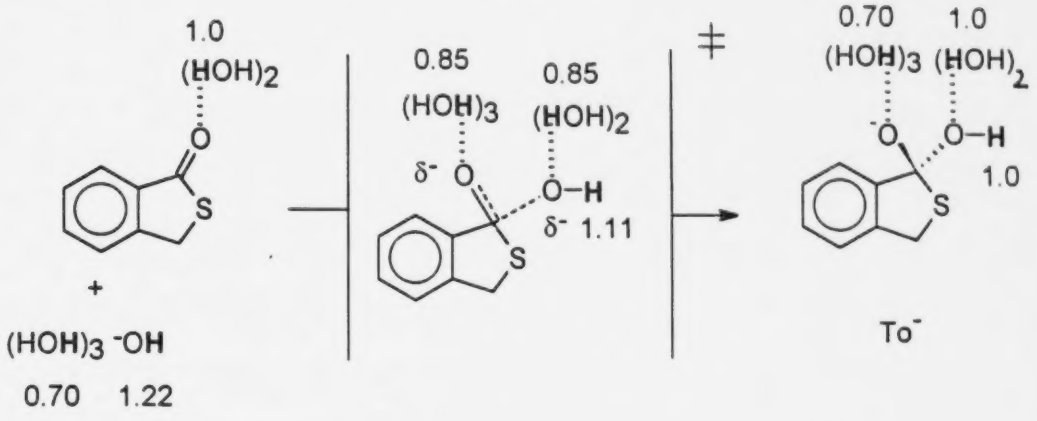


The observation of an inverse isotope effect on C can be used to rule out the process shown in **Scheme 7** which involves a proton in flight in the rate limiting step. Furthermore a process involving reversible formation of To^- with rate limiting decomposition promoted by a second molecule of hydroxide (k_3 term, **Scheme 5**) can also be ruled out since such a process would also require a large and normal SKIE on the constant C^{21h}. Together the lack of ^{18}O exchange and the observed inverse SKIE over the entire [OH^-] domain argue strongly for a mechanism involving rate limiting attack of hydroxide, where the curvature in the k_{hyd} vs. [OH^-] plot arises from a medium effect due to the high electrolyte concentration used in these studies.

The expected SKIE for rate limiting attack of OH^- ($(k_1)_{H_2O/D_2O}$) can be calculated employing fractionation factor analysis as described previously for base catalyzed amide hydrolysis[32] using the method of Schowen and Schowen[33] and modified by the use of Gold's fractionation factors[34] for OH^- and solvating waters. An inverse SKIE equal to that observed for B for thiophthalide (B^{H_2O}/B^{D_2O} = 0.84±0.08) can be calculated if the

position of the transition state (x) is assumed to be 40-50% along the reaction coordinate (**Fig. 4**).

Figure 4. Fractionation factors for the addition of hydroxide to thiophthalide with the transition state 50% along the reaction coordinate (L = H,D; numbers refer to fractionation factors for bold L).

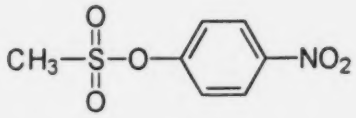


(A transition state that is 60% along the reaction coordinate gives a computed $k_l^{H_2O}/k_l^{D_2O}$ value of 0.90.) The earlier transition state for attack of OL^- on **4** or **5** relative to what is observed for OL^- attack on various toluamides[32a] (x = 0.70, $k_l^{H_2O}/k_l^{D_2O}$ = 0.97) can probably be attributed to the inherent strain present in the ground state of these lactones, which is relieved upon OL^- attack, and to the smaller degree of resonance stabilization in esters relative to amides. These same strain factors would be expected to favour ring opening of To^- over reversal to starting material, and therefore could account for the lack of ^{18}O exchange observed.

3). The third and most likely explanation for the curvature in the k_{hyd} vs. [OH^-] plot relates to medium and/or ion pairing effects. Because of the high electrolyte

concentrations used in these studies (μ = 3M), activity coefficient effects and ion-pairing effects that can normally be disregarded in dilute solutions, may become significant.

In a recent paper, Pregel and Buncel[35] have observed upward curvature in a plot of k_{obs} vs. $[KOEt]_T$ for the ethanolysis of *p*-nitrophenyl methanesulfonate **(12)**.

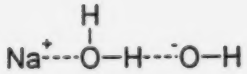


(12)

These authors accounted for the curvature by a mechanism whereby both free and ion paired ethoxide react with ester according to eq. **(23)**, where $k_{ip} > k_{EtO^-}$.

(23) $k_{obs} = k_{EtO^-}[EtO^-] + k_{ip}[KOEt] = (k_{EtO^-}\alpha_{EtO^-} + k_{ip}\alpha_{ip})[KOEt]_T$

As the stoichiometric concentration of KOEt increases the fraction of KOEt in the ion paired form (α_{ip}) should also increase leading to curvature in k_{obs}. While ion-pairing is expected to be more prominent in EtOH than in water, it is known that ion pairing occurs in aqueous solutions of Li, Na and K hydroxides at concentrations of MOH exceeding 1M[36]. Therefore our results could also be explainable by such a scheme if the ion paired hydroxide is inherently more reactive than free hydroxide. Robinson and Harned[37] have suggested that concentrated solutions of alkali metal hydroxides contain solvent bridged ion pairs **(13)**.

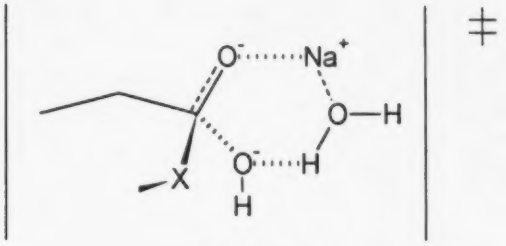


(13)

The chemical reactivity of such a species could be enhanced relative to free hydroxide if a 6 membered ring transition state could be formed wherein the Na^+ ion is able to interact

with the developing charge on the carbonyl oxygen as OH^- attacks the carbonyl carbon as shown below.

Scheme 8:



X = O, S

Alternatively the upward curvature can be explained by an inappropriate correlation of k_{hyd} with $[OH^-]$ in this highly basic medium. Kaiser and Coworkers[38] have studied the hydrolysis of various sulfate and sulfonate esters in highly basic media and have found that the pseudo first order rate constant, k_{obs}, is more correctly correlated with the acidity function H_ according to eq.**(24)**.

(24) $$k_{obs} = k_2°K_w(a_w10^{H-})$$

(here $k_2°$ = the apparent second order rate constant for reaction of OH^- with ester in an ideal dilute solution, K_w = autoprotolysis constant for water, and a_w = activity of water; see Appendix 1-1 for a derivation). A linear correlation of k_{obs} with a_w10^{H-} indicates that the rate of hydrolysis of substrate has a first order dependence on $[OH^-]$[39], while the presence of a second order in $[OH^-]$ term in the rate law will lead to a higher order dependence of k_{obs} on a_w10^{H-}. Shown in **Fig. 5** is a plot of k_{hyd} vs. both $[OH^-]$ and a_w10^{H-} for thiophthalide **(4)**. In constructing the latter curve we have used literature values for a_w[40] and H_ [41] which are only strictly correct at [NaOH] = 3M. Values of a_w and H_ for the other sodium hydroxide concentrations have been estimated as outlined in **Table 4**.

Table 4. Estimated Acidity Function (H_-) and Activity of Water (a_w) Values for NaOH Solutions used in the Hydrolysis of Thiophthalide (**4**).

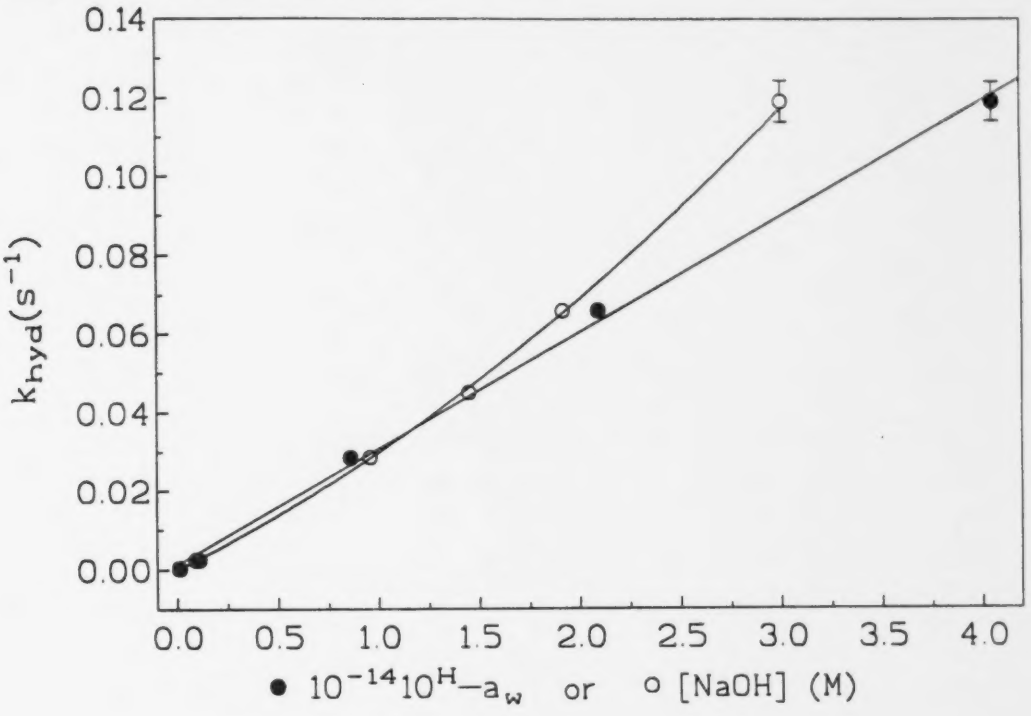
[NaOH] (M)[a]	H_-[b]	a_w[c]	$10^{-14}(10^{H_-})a_w$	$k_{obs}(s^{-1})$
0.0106	12.01	0.9038	0.00922	3.23×10^{-4}
0.108	13.01	0.9038	0.0922	2.47×10^{-3}
0.956	13.98	0.8989	0.8584	0.0284
1.92	14.37	0.8939	2.0955	0.0658
3.01	14.66	0.8884	4.06	0.119

[a] Ionic strength of all solutions adjusted to $\mu = 3.0$ with KCl.

[b] Ref. 41.The values of H_- taken from the literature are for aqueous solutions containing NaOH only. Our solutions also contained KCl for constant ionic strength.

[c] Ref. 40. For the 0.0106 and 0.108 M NaOH solutions a_w was approximated as that of a 3M KCl solution. For the 0.956 and 1.92 M NaOH solutions a_w was estimated from linear interpolation between the values $a_w = 0.9038$ (3M KCl) and $a_w = 0.8884$ (3M NaOH).

Figure 5. Plot of k_{hyd} vs. [NaOH] (O) and $10^{-14}a_wH_-$ (●) for the hydrolysis of thiophthalide **(4)** in H_2O, T = 25°C, μ = 3.0 (KCl).



As shown in **Fig. 5**, the plot of k_{hyd} vs. a_w10^{H-} is indeed linear suggesting that the hydrolysis rate constant involves a first order dependence on [OH-]. Although this analysis was not carried out for phthalide we assume that the curvature in that case can also be attributed to an incorrect correlation of k_{hyd} with [OH-].

(B) Hydrolysis and O^{18} Exchange of Methyl *o*-Methoxybenzoate.

Methyl *o*-methoxybenzoate is the only reported case in the literature of a simple carboxylic ester hydrolyzing with a rate law containing both first and second order terms in hydroxide[22]. Khan and Olagbemiro reported significant curvature in the plot of k_{obs} vs.

[OH⁻] for the hydrolysis of this ester over the NaOH range 0.005-0.2 M (T = 35°C, μ = 2.0). They have postulated that the C[OH⁻]² term (eq. **(21)**) is due to the existence of a dianionic tetrahedral intermediate on the hydrolysis pathway, implying that breakdown of To⁻ must be rate limiting. We have reinvestigated the hydrolysis of **7** under conditions identical to those reported by Khan and find only slight curvature in the k_{hyd} vs. [OH⁻] plot so that the data can be adequately fit over the [NaOH] range 0.005-1.0 M using simple linear regression.

We have also carried out ^{18}O exchange experiments on ~ 50% ^{18}O labelled **7** in 0.0491 and 0.0052 M NaOH. When ester was recovered from the hydrolytic medium after up to two half times of hydrolysis there was no significant change in ^{18}O content from time zero. Providing that the O's of the tetrahedral intermediate are protonically equilibrated we can conclude that there is no significant reversal of the tetrahedral intermediate back to starting material. Thus from both hydrolysis and ^{18}O exchange studies it appears that **7** hydrolyzes in alkaline media with rate limiting attack of OH⁻ and that Khan's claim that this ester hydrolyze through a dianionic tetrahedral intermediate is unjustified.

(C) Hydrolysis and ^{18}O Exchange of Ethyl Benzoate and Ethyl Thiobenzoate.

In order to further investigate the partitioning of To⁻ formed during the hydrolysis of thiol esters in base we have undertaken the hydrolysis and ^{18}O exchange of ethyl thiobenzoate **(6)**. This thiol ester was chosen since the corresponding O ester, ethyl benzoate, had already been shown to undergo ^{18}O exchange in basic media with a partitioning ratio of $k_{ex}/k_{hyd} = 0.079$ (25°C)[6].

The k_{hyd} vs. [OH^-] plots for ethyl benzoate and ethyl thiobenzoate (T = 25°C, μ = 2.0 (KCl)) showed about the same degree of curvature as observed for phthalides **4** and **5**, which we have attributed to a medium effect. The oxygen and thiol esters were found to hydrolyze with almost identical rate constants (B = 0.0341 and B = 0.0321, respectively), however thiol ester **6**, in contrast to the O ester, does not exhibit ^{18}O exchange during its hydrolysis. This indicates that k_1 is the rate limiting step for the base catalyzed hydrolysis of **6**.

Using the partitioning ratios $k_{-1}/k_2 = 0.16$ for ethyl benzoate[6], and $k_{-1}/k_2 \approx 0$ for ethyl thiobenzoate, and given that k_{hyd} (O ester) $\approx k_{hyd}$ (S ester) where $k_{hyd} = \dfrac{k_1[OH^-]}{k_{-1}/k_2 + 1}$, the ratio of rate constants for attack of OH^- on S and O esters can be calculated as: $k_1^S/k_1^O = 0.86$. Therefore the thiol ester appears to be slightly less susceptible to attack by OH^- than the O ester. This observation again raises the question as to the degree of resonance interaction present in S esters compared to O esters. A number of studies[19] have shown that thiol esters behave in many respects like aldehydes or ketones (ie. reduction by $NaBH_4$, ^{13}C chemical shifts of carbonyl C, carbonyl stretching force constants and chemical reactivity patterns) supporting the idea that resonance structure **B** is not a strong contributor in thiol esters because of poor C2p-S3p orbital overlap[19].

A **B**

However other investigators have concluded that resonance stabilization of thiol esters is actually greater than for O esters. Wolfe and coworkers[42] have performed quantum mechanical calculations showing that the barrier to rotation in protonated thioformaldehyde is considerably greater than that in protonated formaldehyde and have concluded that the π donating ability of S towards a cationic center is greater than that of O. Noe[43] has experimentally determined the barrier to rotation about the C(O)-S bond in thioacetic acid, and found it to be comparable to that of amides. He has concluded that π bonding ability decreases in the order $N \geq S > O$. The results of our study, demonstrating similar rates of attack of OH^- on O and thiol esters, can provide no definitive answer to this question since we have no information on the enthalpic and entropic contributions to k_1 for the two esters. The question of thiol ester resonance therefore remains as an unresolved question.

Conclusions

The present results indicate that the hydrolysis of esters such as **4** and **5** in highly alkaline media show significant upward curvature in the k_{hyd} vs $[OH^-]$ plots. Yet, no ^{18}O-exchange is observed in the recovered starting material. We have attempted to facilitate, using the bifunctional buffer system HCO_3^-/CO_3^{-2}, the protonic equilibration of the oxygens in To^- only to observe that no ^{18}O-exchange is evident in the recovered starting materials. Solvent kinetic isotope effect studies also do not provide evidence that the apparent upward curvature in the k_{hyd} vs $[OH^-]$ plots derives from a second order in $[OH^-]$ term since such would imply a substantial normal $\left(k_{hyd}\right)_{H_2O/D_2O}$ effect on the latter stemming from a proton in flight. Despite the limitations imposed by our negative findings, the best current explanation for the upward curvature in the plots invokes medium and/or ion pairing effects in the hydrolysis. Indeed, the k_{hyd} values for thiophthalide correlate nicely with H_- in a way that indicates only one OH^- is involved in the hydrolysis. A reinvestigation of the hydrolysis of **7** fails to provide evidence for the reported[22] prominent $[OH^-]^2$ term. It therefore seems likely that the hydrolysis of simple carboxylic acid esters proceeds through transition states involving a single OH^-, and there is, at present, no compelling evidence for second order terms in any case.

Appendix 1-1 - Supplementary Material to Part 1 - Chapter 1

Table A-1. ^{18}O-Exchange Data, Thiophthalide, T = 25°C, μ = 3.0 (KCl)

Medium	Time Reaction Terminated[a]	Avg. % ^{18}O in Recovered Ester[b]
0.0106 M NaOH	0	51.48±0.24
	$t_{1/2}$	51.08±0.25
	1.5 $t_{1/2}$	53.82±0.15
	2 $t_{1/2}$	52.33±0.73
$KHCO_3/K_2CO_3$	0	53.70±0.40
[Buffer] = 0.8 M	$t_{1/2}$	52.75±0.76
pH = 9.56	1.5 $t_{1/2}$	51.87±0.83
	2 $t_{1/2}$	53.42±0.43

[a] $t_{1/2}$ refers to 1 half time for hydrolysis. At 0.0106 M NaOH, $t_{1/2}$ = 35.8 min.; at pH = 9.56, $t_{1/2}$ = 129 h.

[b] Average $\%^{18}O$ ± 1 standard deviation, determined from ~ 20 low resolution mass spectral scans of this sample.

Table A-2. ^{18}O-Exchange Data, Phthalide, T = 25°C, μ = 3.0 (KCl)

Medium	Time Reaction Terminated[a]	Avg. % ^{18}O in Recovered Ester
0.0106 M NaOH	0	54.95±0.59
	0.5 $t_{1/2}$	54.85±0.26
	$t_{1/2}$	55.47±0.17
	1.5 $t_{1/2}$	54.93±0.13
$KHCO_3/K_2CO_3$	0	55.00±0.24
[Buffer] = 0.8 M	$t_{1/2}$	54.92±0.82
pH = 9.81	1.5 $t_{1/2}$	55.03±2.20
	2 $t_{1/2}$	52.97±1.33

[a] $t_{1/2}$ refers to 1 half time for hydrolysis. At 0.0106 M NaOH, $t_{1/2}$ = 261 s; at pH = 9.81, $t_{1/2}$ = 24h.

Table A-3. ^{18}O-Exchange Data, Methyl *o*-Methoxybenzoate, T = 35°C, μ = 2.0 (KCl)

Medium	Time Reaction Terminated[a]	Avg. % ^{18}O in Recovered Ester
0.00518 M NaOH[a]	0	46.36±0.18
	$t_{1/2}$	46.32±0.25
	1.5 $t_{1/2}$	46.40±0.22
0.0491 M NaOH[b]	0	46.36±0.18
	$t_{1/2}$	46.12±0.58
	1.5 $t_{1/2}$	45.72±0.35
	2 $t_{1/2}$	45.69±0.42

[a] $t_{1/2}$ hydrolysis = 35.0 min

[b] $t_{1/2}$ hydrolysis = 208 s

Table A-4. ^{18}O-Exchange Data, Ethyl Thiobenzoate, T = 25°C, μ = 2.0 (KCl)

[NaOH] (M)	Time Reaction Terminated[a]	Avg. % ^{18}O in Recovered Ester
0.00499[a]	0	46.19±0.40
	2.5 $t_{1/2}$	45.88±0.54
0.010[b]	0	46.41±0.10
	$t_{1/2}$	46.29±0.21
	2 $t_{1/2}$	46.61±0.19
	2.5 $t_{1/2}$	46.72±0.08

[a] $t_{1/2}$ hydrolysis = 78.5 min.

[b] $t_{1/2}$ hydrolysis = 36.9 min.

Derivation of Equation (24).

The H_ acidity function is defined as[41b]:

$$(1') \quad H_- = pK_A - \log\frac{[HA]}{[A^-]} = -\log\frac{a_{H_3O^+} f_{A^-}}{a_w f_{HA}}$$

where a_i = activity of species i and f_i = activity coefficient of species i (a_w = activity of water).

Eq. (1') is derived from the thermodynamic equilibrium constant $K_A = a_{H_3O^+} a_{A^-} / a_{HA} a_w$ for dissociation of an indicator acid HA. Eq. (1') can be rewritten as :

$$(2') \quad H_- = -\log h_-$$

where

$$(3') \quad h_- = \frac{a_{H_3O^+} f_{A^-}}{a_w f_{HA}} = \frac{K_w a_w f_{A^-}}{a_{OH^-} f_{HA}}$$

and where K_w = autoprotolysis constant of water = $a_{H_3O^+} a_{OH^-} / a_w^2$.

For the bimolecular reaction

$$S + OH^- \xrightarrow{k_2} P$$

the rate law is given by:[44]

$$(4') \quad \frac{d[P]}{dt} = k_2^\circ [S][OH^-] \frac{f_{OH^-} f_s}{f_\ddagger}$$

$$= k_{obs}[S]$$

where

$$(5') \quad k_{obs} = k_2^\circ a_{OH^-} \frac{f_s}{f_\ddagger}$$

and $k_2°$ = the apparent second order rate constant for reaction of substrate with OH^- in ideal dilute solution. From eq. (3′) above we have

(6′) $$\frac{a_{OH^-} f_{HA}}{f_{A^-}} = \frac{K_w a_w}{h_-}$$

If the equality $\frac{f_s}{f_\ddagger} \approx \frac{f_{HA}}{f_{A^-}}$ holds, then eq. (5′) can be rewritten as:

(7′) $$k_{obs} = \frac{k_2° K_w a_w}{h_-}$$

or

(8′) $$\log k_{obs} = \log(k_2° K_w) + \log a_w - \log(h_-)$$

$$= \log(k_2° K_w) + \log a_w + H_-$$

or

(24) $$k_{obs} = k_2° K_w (a_w 10^{H_-})$$

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b). ^{18}O exchange of **3** carried out in 40% Dioxane/ H_2O. See ref. 7(a).

In consideration of these results it should be noted that k_{ex}/k_{hyd} would be expected to increase upon passing from a solvent containing significant amounts of a nonpolar additive to pure H_2O.

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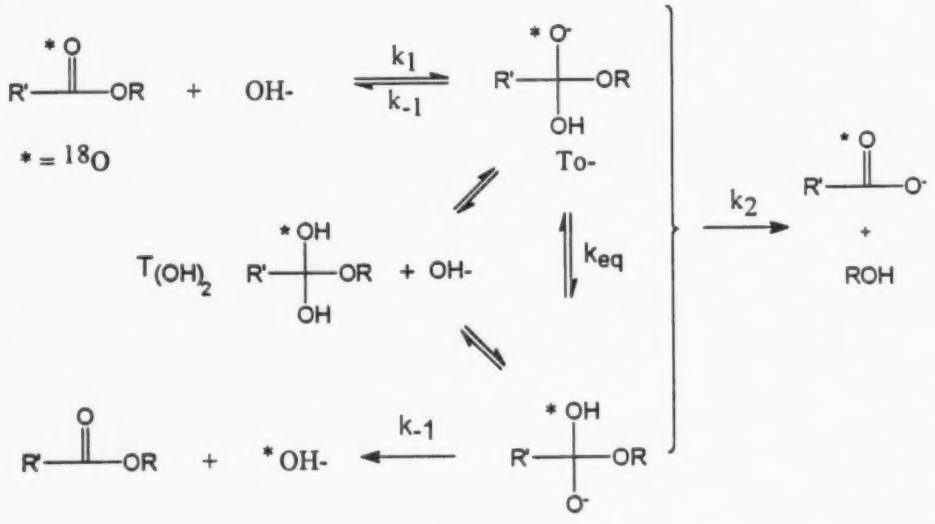
Part 1 - Chapter 2

Base Catalyzed Hydrolysis and ^{18}O Exchange of Ethyl and Isopropyl Toluoate in H_2O and D_2O Media*

Introduction

As discussed in Chapter 1, exchange of ^{18}O out of ester labelled in the carbonyl oxygen should occur concurrently with hydrolysis if the tetrahedral intermediate, To$^-$, is reversibly formed. If the processes for exchange and hydrolysis are those outlined in **Scheme 1**,

Scheme 1:



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* A version of this chapter has been published. Kellogg, B.A.; Tse, J.E.; Brown, R.S. *J. Am. Chem. Soc.* **1995**, *117*, 1731-1735.

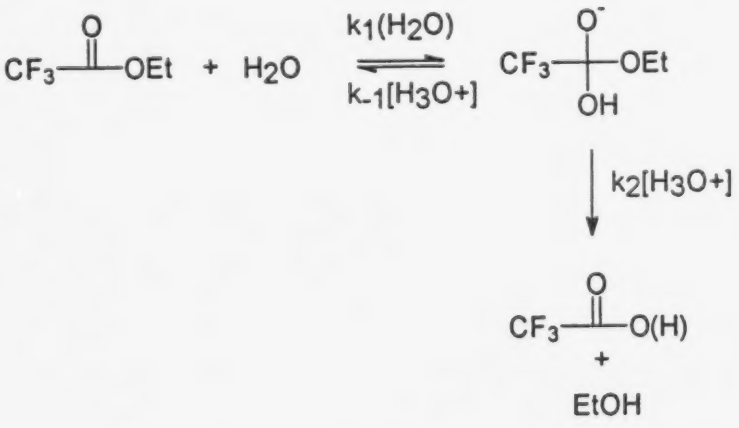
then the expressions for k_{ex} and k_{hyd} are $k_{ex} = \frac{k_1 k_{-1}[OH^-]}{2(k_{-1} + k_2)}$; $k_{hyd} = \frac{k_1 k_2 [OH^-]}{k_{-1} + k_2}$; and $k_{ex}/k_{hyd} = k_{-1}/2k_2$ (see Chapter 1 for derivations). In this scheme, k_{eq}, corresponds to the rate constant for proton transfer between the 2 oxygens of the tetrahedral intermediate and may correspond to a single step or multi-step process. The expressions for k_{ex} and k_{ex}/k_{hyd} have been derived assuming that the oxygens of To^- are completely protonically equilibrated so that there is equal probability of expelling $^{16}OH^-$ or $^{18}OH^-$ upon reversal of To^- to starting material. Therefore k_{ex}/k_{hyd} will only be a true measure of the partitioning of the tetrahedral intermediate between starting materials and products if the above assumption is true. If in fact the rate of proton transfer is slow relative to the rate of decomposition of To^- ($k_{eq} \leq (k_{-1} + k_2)$), then k_{ex}/k_{hyd} can only provide a lower limit on the ratio $k_{-1}/2k_2$ since reversal will be accompanied by exchange less than 50% of the time. It is therefore important to establish whether the assumption of protonic equilibration is correct if we want to use ^{18}O exchange data in a quantitative sense to measure partitioning ratios.

In the case of ester hydrolysis in base a number of authors have expressed doubt as to the validity of this assumption. Moffatt and Hunt[1] first suggested that protonic equilibration might not be complete based on experiments showing differing solvent effects on the rates of acidic and basic hydrolysis of alkyl esters. Bender and Thomas[2] suggested the same thing in an effort to account for the observed variation in k_{ex}/k_{hyd} with substituent for a number of *p*-substituted methyl benzoates. However the results on which this discussion was predicated could not be reproduced by other workers[3]. Shain and

Kirsch[3] also discuss the question of oxygen equilibration and suggest that the lack of such might explain the observed order of partitioning ratios (k_{ex}/k_{hyd}) for a number of benzoate esters. That is, the trend in k_{ex}/k_{hyd} observed for a series of benzoate esters could not be simply explained based on apparent leaving group ability, so these authors suggested that for some or all of these esters protonic equilibration of To$^{\pm}$ might be incomplete.

In their study of the neutral hydrolysis of ethyl trifluoroacetate, Bender and Heck[4] actually provided evidence from solvent kinetic isotope effect (SKIE) studies that protonic equilibration was incomplete. The mechanism of the neutral hydrolysis of this ester is given below:

Scheme 2:

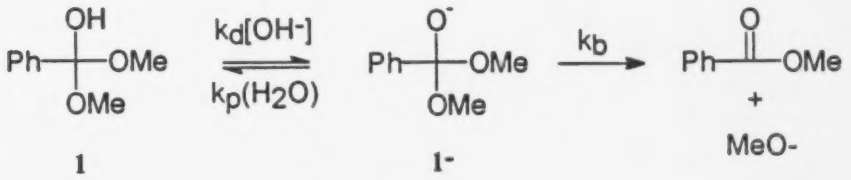


For such a mechanism involving a symmetrically catalyzed partitioning of the tetrahedral intermediate between starting material and products, the expression for k_{ex}/k_{hyd}, provided that proton transfer is fast within To$^{\pm}$, is again given by: $k_{ex}/k_{hyd} = k_{-1}/2k_2$. If this equation is valid an isotope effect of unity is expected on k_{ex}/k_{hyd} since k_{-1} and k_2 both involve general acid catalyzed expulsion of OR (R = H or alkyl group) so that their respective

isotope effects should cancel. It was found however that the ratio k_{ex}/k_{hyd} decreased significantly in D_2O with a measured SKIE $(k_{ex})_{(H_2O/D_2O)}$ of ~ 3. The authors therefore concluded that proton transfer within the tetrahedral intermediate must be kinetically significant for exchange.

McClelland and coworkers[5] have been able to directly measure the rate of OH^- catalyzed decomposition of hemiorthoester **1**, which corresponds to the tetrahedral intermediate of a transesterification reaction.

Scheme 3:

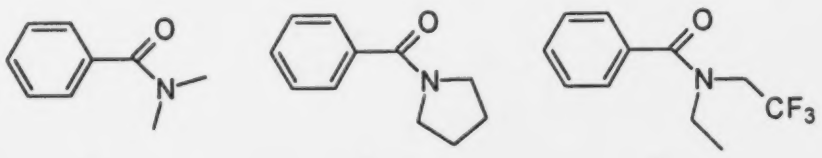


1 **1⁻**

From pH-rate studies the rate of decomposition of $\mathbf{1}^-$ (k_b) was found to be $9x10^6$ s^{-1}. A value for the rate of protonation of $\mathbf{1}^-$ (k_p) could also be estimated from the kinetic data by assuming that the deprotonation of **1** occurs at the diffusion limit[6] ($k_d \approx 10^{10}$ $M^{-1}s^{-1}$). From this analysis they determined that the rate of decomposition of $\mathbf{1}^-$ (k_b) may be competitive with protonation (k_p was estimated to lie in the range of ~ $2x10^6$ - $5x10^7$ s^{-1}). These studies obviously have implications for the question of the protonic equilibration of To^- formed during ester hydrolysis in base. If the proton transfer in To^- occurs through a protonation - deprotonation mechanism (i.e. through $T_{(OH)_2}$, **Scheme 1**) then in order for the tetrahedral intermediate to be completely protonically equilibrated we require that $k_p >> (k_{-1} + k_2)$ where k_p = rate constant for protonation of To^- by H_2O. If the hemiorthoester

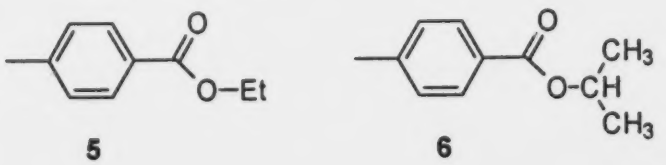
anions of McClelland's study provide good models of the anionic tetrahedral intermediates formed during ester hydrolysis in base then these results would suggest that the lifetime of the tetrahedral intermediate may be comparable with, or shorter than, the time required to attain proton equilibrium, if proton transfer occurs through $T_{(OH)_2}$.

Brown and coworkers[7] have recently undertaken solvent kinetic isotope effect studies on the exchange and hydrolysis of toluamides under basic conditions. If proton transfer between the oxygens of the tetrahedral intermediate is rate limiting for exchange then a large normal SKIE on k_{ex} would be expected. For all three tertiary toluamides shown below, an inverse isotope effect on k_{ex} was found ($(k_{ex})_{H_2O/D_2O} < 1.0$) suggesting that proton transfer is not rate limiting for exchange.



2 **3** **4**

We have undertaken similar studies on the hydrolysis and exchange of two esters, ethyl toluoate (**5**) and *iso*propyl toluoate (**6**), whose close analogs, the corresponding benzoate esters, are known to exhibit exchange[3]. These studies were undertaken in order to further probe the question of protonic equilibration of the anionic tetrahedral intermediate formed during the hydrolysis of esters in base.



5 **6**

It will be shown that the measured isotope effect on k_{ex} is not significantly different from unity in either case suggesting that the oxygens of the anionic tetrahedral intermediate are protonically equilibrated.

Experimental

(A) Syntheses.

Ethyl toluoate (**5**) and *iso*propyl toluoate (**6**) were obtained by dropwise treatment of excess alcohol with neat toluoyl chloride followed by heating to reflux for 7 h (in the case of **5**) and 21 h (in the case of **6**). After standard workup procedures, the esters were purified by distillation.

The ^{18}O labelled esters were obtained by the procedure described below for labelled **6**. To 0.855 mL (0.0065 mol) of toluoyl chloride in a 25 mL flask was added 0.14 mL (0.0077 mol) of 97% $H_2{}^{18}O$. This mixture was allowed to stand at room temperature for 17 h and then treated with 25 mL of $SOCl_2$. After heating at 70°C for 2 h, the excess thionyl chloride was removed by distillation. The ^{18}O labelled toluoyl chloride was distilled (Kugelrohr) and then treated with 3 mL of *iso*propanol followed by heating at reflux for 48 h. At the end of this time 25 mL of ether was added to the solution. This solution was extracted with 2 x 5 mL of 10% K_2CO_3, and then 5 mL of saturated NaCl. The solution was dried ($MgSO_4$), filtered, and the residue purified by microdistillation. IR ($CHCl_3$ cast) 2981, 1715 (^{16}O=C), 1685 (^{18}O=C), 1612, 1276 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 2 H), 7.21 (d, 2 H), 5.23 (m, 1 H), 2.41 (s, 3 H), 1.39 (d, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.63, 21.99, 68.09,128.30, 128.99, 129.57, 143.28, 166.18 (^{18}O=C), 166.22 (^{16}O=C); Exact mass calcd. for $C_{11}H_{14}{}^{18}O_1{}^{16}O_1$, 180.10362; found, 180.10348.

The spectral parameters for ^{18}O labelled **5** are: IR ($CHCl_3$ cast) 2982, 1719 (^{16}O=C), 1688 (^{18}O=C), 1613, 1276 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.96 (d, 2 H), 7.24 (d, 2 H), 4.48 (q, 2 H), 2.42 (s, 3 H), 1.40 (t, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.93, 21.09, 60.24, 127.54, 128.80, 129.53, 142.91, 166.05 (^{18}O=C), 166.10 (^{16}O=C). Exact mass calcd. for $C_{10}H_{12}{}^{18}O_1{}^{16}O_1$, 166.08797, found,166.08811.

(B) Hydrolysis Kinetics.

(i) H_2O. All base solutions were made by dilution of 19 M NaOH under CO_2 free conditions (drybox) using CO_2 free H_2O (Osmonics-Aries® water purification system). Ionic stength was maintained at 0.1 (NaCl). NaOH solutions were titrated with standardized 0.0997 M HCl (Aldrich) using bromothymol blue as an indicator, or with potassium hydrogen phthalate solution using phenolphthalein indicator.

The hydrolysis kinetics of **5** and **6** were monitored at 25°C, μ = 0.1 (NaCl) by observing the rate of diminution of the uv bands at 248 (**5**) and 250 (**6**) nm using an OLIS® modified Cary 17 spectrophotometer. Cuvettes were charged with 3 mL of the appropriate base solution under CO_2 free conditions and allowed to thermally equilibrate in the spectrometer cell holder for 15 minutes. Reactions were initiated by injecting 125 μL of a 1.75 mM (for **5**) or 1.85 mM (for **6**) solution of the ester in DME into the aqueous base (final concentration of esters 6 and 5 x 10^{-4} M respectively). Reactions were followed in triplicate to at least 5 half times at base concentrations of ~ 0.01 and 0.1 M.

ii) D_2O. NaOD solutions of 0.0103 M and 0.0960 M were made by adding Na metal to ice cooled D_2O, μ = 0.1 (NaCl), under an atmosphere of Ar. Exact base concentrations were determined by titration against standardized HCl (0.0997 N) using bromothymol blue as an indicator, or against potassium hydrogen phthalate using phenolphthalein. Hydrolysis kinetics in D_2O were performed as above.

(C) ^{18}O Exchange.

These experiments were conducted following protocols established before[8] for ^{18}O exchange accompanying base promoted amide hydrolysis. Care was taken to ensure that the esters (particularly **6**) were soluble in the medium. A typical experiment is described below for **6**.

To 700 mL of the appropriate base solution (0.0102 M or 0.095 M NaOH, μ = 0.1 (NaCl)) in a 1000 mL volumetric flask (equilibrated in a 25°C thermostated bath) was added 30 mL of a 0.0125 M solution of **6** in DME. The flask was inverted several times and allowed to stand in the 25°C bath for the appropriate time (up to 3 half times of hydrolysis) during which 100 mL fractions of the reaction mixture were removed at various times and quenched with 88 µL (or 0.86 mL for 0.095 M NaOH) of 7.4 M H_3PO_4. The final pH was 6-7. The solution was extracted with 3 x 30 mL of distilled CH_2Cl_2. The combined CH_2Cl_2 extracts were dried, filtered, and the volatiles removed. To the residue were added 10 drops of CH_2Cl_2; this was then transferred to a vial for low resolution mass analysis.

The ^{18}O exchange for **5** and **6** was also conducted in D_2O solutions (0.0960 M NaOD, μ = 0.1 (NaCl) for **6**; and 0.0103 M NaOD, μ = 0.1 (NaCl) for **5**) using the above protocol.

In each of the above cases, ^{18}O analyses were conducted in duplicate at 5 to 6 times up to 3 $t_{1/2}$ hydrolysis. The ^{18}O content in recovered ester was determined as % $^{18}O = 100 \times I_{M^+2} / (I_{M^+} + I_{M^+2})$ where I is the intensity of the M^+ and M^+2 peaks: 20-25 scans of these peaks were taken for each sample. The % ^{18}O vs time data are given in **Tables A-1 to A-7**, Appendix 1-2.

Results

Given in **Table 1** are the hydrolysis rate constants for **5** and **6** in H_2O and D_2O media at 25°C. The solutions contained 4% DME for solubility of the esters so that the hydrolysis and ^{18}O exchange experiments (the latter require higher [ester]) could be directly compared. In comparing the hydrolytic data in H_2O and D_2O, inverse deuterium kinetic isotope effects (DKIE) for the hydrolysis of **5** and **6** are observed, $\left(k_{hyd}\right)_{H_2O/D_2O}$ = 0.75±0.27 and 0.81±0.13 respectively.

^{18}O exchange accompanying the hydrolysis was determined by low resolution mass analysis of ester recovered from the hydrolytic media at various times up to 3 $t_{1/2}$ hydrolysis. The k_{ex} rate constants for **5** and **6** in basic media are given in **Table 2**. In the case of the exchange studies in D_2O, the low solubility of the esters required large volumes of D_2O, so only a single base concentration was investigated for each ester. Using the average values for the k_{ex} and k_{hyd} given in **Tables 1** and **2**, the following ratios can be computed: for **5** $\left(k_{ex}/k_{hyd}\right)_{H_2O}$ = 0.068±0.005, $\left(k_{ex}/k_{hyd}\right)_{D_2O}$ = 0.052±0.009; for **6** $\left(k_{ex}/k_{hyd}\right)_{H_2O}$ = 0.15±0.01, $\left(k_{ex}/k_{hyd}\right)_{D_2O}$ = 0.14±0.01.

Table 1. Rate Constants for Hydrolysis of **5** and **6** in Base, T = 25°C, μ = 0.1 (NaCl).

Ester	[NaOL] (M)	k_{obs} (s^{-1})[a]	k_{hyd} (M^{-1} s^{-1})[b]
5	0.00979	$(1.42\pm0.01)\times10^{-4}$	
			0.015±0.001
5	0.0912	$(1.39\pm0.03)\times10^{-3}$	
5	0.00989 (D_2O)	$(1.39\pm0.06)\times10^{-4}$	
			0.020±0.007
5	0.0922 (D_2O)	$(1.81\pm0.07)\times10^{-3}$	
6	0.00956	$(3.1\pm0.1)\times10^{-5}$	
			$(3.37\pm0.12)\times10^{-3}$
6	0.0922	$(3.10\pm0.04)\times10^{-4}$	
6	0.00989 (D_2O)	$(4.64\pm0.09)\times10^{-5}$	
			$(4.17\pm0.64)\times10^{-3}$
6	0.0922 (D_2O)	$(3.87\pm0.09)\times10^{-4}$	

[a] Pseudo first order rate constant; average of 2-3 determinations, error are ±1 SD in the average k_{obsd}; contains 4% DME.

[b] k_{hyd} is second order rate constant computed from linear regressions of plots of k_{obsd} vs [OH^-]; quoted errors are ±2 SD of the slope.

Table 2. Rate Constants for ^{18}O Exchange Accompanying Base Hydrolysis of ^{18}O-Labelled **5** and **6**, T = 25°C, μ = 0.1 (NaCl).

Ester	[NaOL] (M)	k_{obs} (s^{-1})[a]	k_{ex} (M^{-1} s^{-1})[b]
5	0.00981	$(1.07\pm0.07)\times10^{-5}$	1.09×10^{-3}
5	0.00981	$(1.02\pm0.09)\times10^{-5}$	1.04×10^{-3}
5	0.00927	$(8.9\pm1.1)\times10^{-6}$	9.6×10^{-4}
			AVG = $(1.02\pm0.14)\times10^{-3}$
5	0.00989 (D_2O)	$(1.04\pm0.02)\times10^{-5}$	$(1.05\pm0.04)\times10^{-3}$
6	0.0913	$(4.82\pm0.25)\times10^{-5}$	5.28×10^{-4}
6	0.092	$(4.33\pm0.15)\times10^{-5}$	4.71×10^{-4}
			AVG = $(4.99\pm0.80)\times10^{-4}$
6	0.0922 (D_2O)	$(5.37\pm0.07)\times10^{-5}$	$(5.82\pm0.16)\times10^{-4}$

[a] Pseudo first order rate constant; k_{obs} determined from linear regression of ln (% ^{18}O) vs. time; 6-8 data points; error is ±1 SD of the slope; contains 4% DME for solubility.

[b] Average k_{ex} is the mean of the individual k_{obs} / [OL^-] values. For **5** and **6** in H_2O, errors in k_{ex} are given as ±2 SD from the mean. In D_2O, since k_{ex} is a single number, the errors are given ±2 SD of the linear regression of ln (% ^{18}O) vs. time.

Discussion

Bender[9] originally investigated ^{18}O exchange accompanying the basic hydrolysis of ethyl and *iso*propyl benzoate, in H_2O. The k_{ex}/k_{hyd} ratios found by him were: ethyl benzoate = 0.20, *iso*propyl benzoate = 0.37. Shain and Kirsch[3] later reported a value of k_{ex}/k_{hyd} = 0.079 for ethyl benzoate in H_2O, which is very close to our value for ethyl toluoate (k_{ex}/k_{hyd} = 0.068±0.005). The value found here for *iso*propyl toluoate (k_{ex}/k_{hyd} = 0.15±0.01) is somewhat lower than Bender's reported value for *iso*propyl benzoate.

Using the average values for k_{ex} given in **Table 2**, solvent kinetic isotope effects near unity were calculated for k_{ex} for both esters with $\left(k_{ex}\right)_{H_2O/D_2O}$ = 0.97±0.14 for **5** and $\left(k_{ex}\right)_{H_2O/D_2O}$ = 0.86±0.14 for **6**. The overall SKIE's on the ratio k_{ex}/k_{hyd} were also found to be close to unity with $\left(k_{ex}/k_{hyd}\right)_{H_2O/D_2O}$ = 1.31±0.25 for **5** and $\left(k_{ex}/k_{hyd}\right)_{H_2O/D_2O}$ = 1.07±0.10 for **6**. The SKIE on k_{ex}/k_{hyd} directly measures the isotope effect on the two transition states leading away from To$^-$ which correspond to the barriers for k_{-1} and k_2 in the case where protonic equilibration is complete. Since the expulsion of HO^- and RO^- are both expected to be uncatalyzed processes, any isotope effects on k_{-1} and k_2 (**Scheme 1**) should effectively cancel and an isotope effect of unity will be expected if $k_{ex}/k_{hyd} = k_{-1}/2k_2$. If, however, proton transfer within To- is rate limiting for exchange the expression for k_{ex}/k_{hyd} becomes[5]: $\frac{k_{ex}}{k_{hyd}} = \left(\frac{k_{-1}}{k_2}\right)\frac{k_{eq}}{k_{-1}+k_2+2k_{eq}}$. In this case a normal kinetic isotope effect will be expected on k_{eq} and thus on the ratio k_{ex}/k_{hyd}. The absence of a strong primary SKIE ($k_{H_2O/D_2O} > 2$) on either k_{ex} or k_{ex}/k_{hyd} tends to argue against any process

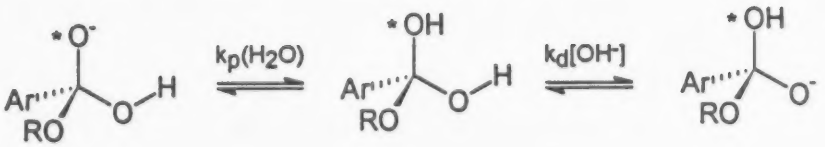
involving a proton in flight being rate limiting for exchange. However the possibility that rate limiting proton transfer is associated with an unusually small KIE due to nonlinearity or asymmetry in the transition state[10] must be considered. To further investigate this possibility a closer examination of the possible mechanisms for proton transfer in To$^-$ is required.

Mechanistic Possibilities. There are three potential mechanisms (**A-C, Scheme 4**) for proton transfer within the tetrahedral intermediate that warrant consideration. These involve, respectively, a direct intramolecular proton transfer through a 4 membered ring transition state (**A**), a stepwise protonation of To$^-$ by H_2O followed by deprotonation by OH^- (**B**), and a concerted proton transfer via one or more bridging water molecules (**C**).

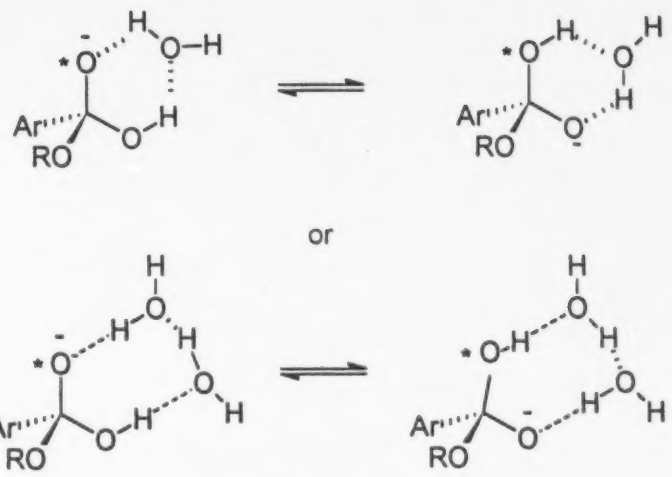
Scheme 4:

(A)

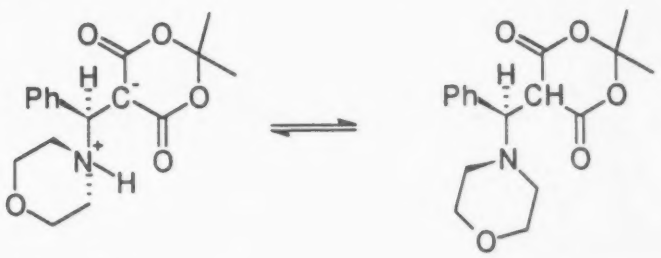
(B)



(C)



Intramolecular proton transfer as depicted in **A** might be expected to proceed with a small DKIE because of the extreme nonlinearity of the T.S [10a,b]. However Gandour has suggested, based on the results of a number of studies[11], that intramolecular proton transfer will only occur readily in systems where it is possible to form transition states of appropriate size to accommodate linear proton transfers (there have been objections raised to this assertion however, *vide infra*). This argues against a direct intramolecular proton transfer as shown in **A**. Bernasconi et. al.[12] have demonstrated the unfavourability of such an intramolecular proton transfer in their studies on the system $7^{\pm} \rightarrow 7^{\circ}$ shown below.

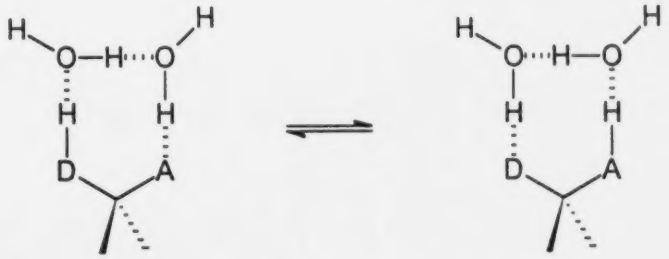


7± **7°**

Proton inventory studies have shown that there are 2-3 protons in flight in the transition state for this proton transfer reaction. Therefore proton transfer must occur through one or two bridging H_2O molecules. A large 1° SKIE of $k_H/k_D = 3.26$ was also observed for this process.

The fact that proton transfer in this system proceeds via a H_2O bridge even though proton transfer between carbanions and N or O acids usually occurs directly[13], attests to the unfavourable nature of a such a 4 membered ring transition state. According to Gandour[11], when proton transfer occurs through a solvent bridge, in systems where the donor and acceptor atoms are separated by 1 carbon atom, the solvent bridge will usually consist of two water molecules since the 8 membered ring transition state so formed can accommodate linear H bonds (**Scheme 5**).

Scheme 5:



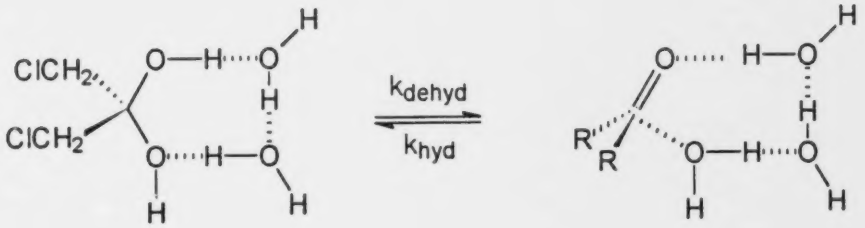
D = donor atom
A = acceptor atom

If this kind of concerted proton transfer is rate limiting for exchange, a large SKIE should be observed since there are 3 protons in flight.

There are a number of examples in the literature of proton transfer occurring via such a mechanism. Grunwald and coworkers[14] and Luz and Meiboom[15] have measured

the exchange of carboxylic acid protons with solvent and interpreted their results in terms of a mechanism involving H^+ transfer within a H bonded complex consisting of one acetic acid molecule and two solvent molecules. Similarly the dehydration of the hydrate of 1,3 dichloroacetone[16] (**Scheme 6**) was suggested to occur through a bridge of two water molecules.

Scheme 6:

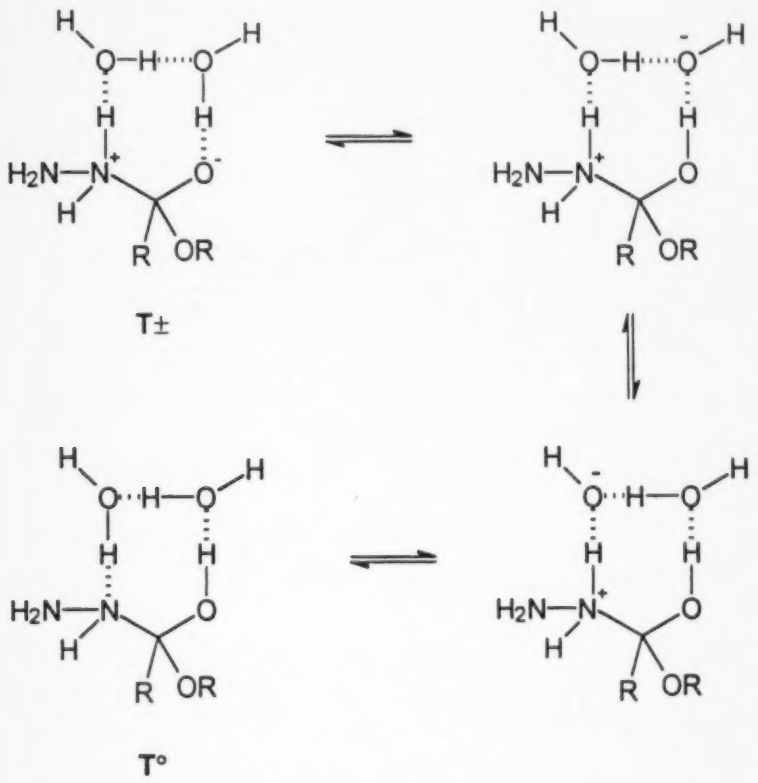


The hydrate shown above is closely analogous to the tetrahedral intermediate formed duing the basic hydrolysis of esters, suggesting that the proton transfer in To^- may also proceed through such an 8 membered ring transition state involving 2 H_2O molecules. On the basis of the observed primary kinetic isotope effect for the hydration reaction ($\left(k_{hyd}\right)_{H_2O/D_2O} = 2.7$), one would also predict a 1° SKIE on the dehydration reaction, and by analogy on proton transfer in To^- if such a process is rate limiting.

A third example of proton transfer proceeding through an 8 membered ring consisting of bridging H_2O molecules was provided by Satterthwait and Jencks[17]. These authors showed that the water catalyzed reaction of hydrazine with alkyl esters involves rate limiting conversion of the zwitterionic tetrahedral intermediate (T±) to the neutral tetrahedral intermediate (T°) with the proton transfer probably occurring through a bridge

of two water molecules (**Scheme 7**). Based on structure reactivity data it was proposed that the proton switch occurs through a stepwise process in this system.

Scheme 7:



Although these examples suggests that proton transfer through 2 bridging water molecules is the most likely mechanism for proton transfer in To˙ it is also possible that tranfer can occur through a single bridging water molecule as discussed by Grunwald[18] and Gandour[11] and again by Menger[19]. Grunwald suggests that in such a case proton transfer could occur in a stepwise fashion where the bridge molecule after the first proton transfer could rotate into proper alignment for a second linear proton transfer (this would correspond to mechanism **B** in **Scheme 4**). Menger[19] however disagrees with Gandour's

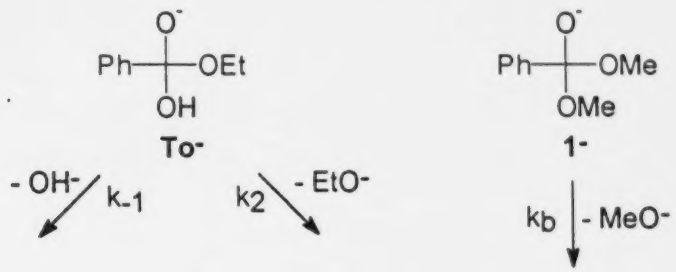
assumption that proton transfers must necessarily proceed through linear transition states. He has provided both computational[20] and experimental evidence[21] suggesting that transition states involving nonlinear proton transfers are energetically accessible. On this basis he suggests that the 1,3-proton transfer between heteroatoms in a tetrahedral intermedate might take place in a concerted fashion through a single bridging water molecule (although he doesn't specifically rule out the possibility of the involvement of two water molecules).

Therefore of the three mechanisms proposed for proton transfer in To^-, **B** and **C** remain as the most likely candidates for this process. Since both of these processes would be expected to exhibit significant 1° kinetic isotope effects if rate limiting for exchange (especially the concerted processes which involve two or more protons in flight), we conclude, based on the lack of such an isotope effect, that proton transfer within To^- is not rate limiting for exchange and that the tetrahedral intermediates are protonically equilibrated.

How can the results of this study be reconciled with those of McClelland already described involving breakdown of hemiorthoester anions? As McClelland states, the fact that k_p and k_b (**Scheme 3**, Introduction) are on the same order of magnitude argues that **1** and $\mathbf{1}^-$ (and by analogy $T_{(OH)_2}$ and To^-) are not in full protonic equilibrium. However the unit isotope effect on k_{ex} measured in our studies for esters **5** and **6** requires that if O equilibration is proceeding by the pathway $T_o^- \xrightarrow{k_p(H_2O)} T_{(OH)_2} \xrightarrow{k_d[OH^-]} T_o^-$ then k_p must be sufficiently larger than k_b (the rate of decomposition of To^-) so that it is not rate limiting. One could possibly argue that the rate of breakdown of To^- will be sufficiently

slower than the rate of breakdown of the hemiorthoester anion, 1^- (**Scheme 8**) such that k_p is now >> k_b (where $k_b = k_{-1} + k_2$ in the case of To^-).

Scheme 8:



This is perhaps not improbable when considering the fact that To^- formed from attack of OH^- on methylbenzoate expells CH_3O^- much more readily than HO^- ($k_{-1} = 0.07k_2$)[3] even though CH_3O^- and HO^- have approximately identical pK_A's.

An alternative and more satisfying conclusion is that proton transfer within To^- occurs not via a stepwise mechanism involving the intermediacy of $T_{(OH)_2}$, but instead by a concerted proton transfer through two bridging water molecules. This kind of transfer could be sufficiently faster than the stepwise process so that now proton transfer becomes much faster than breakdown. This explanation appears the most appealing to us because of the ample amount of evidence in the literature for such a proton transfer mechanism.

Conclusion

The alkaline hydrolysis and exchange of ethyl and *iso*propyl toluoate in H_2O and D_2O media were carried out and it was shown that the SKIE on k_{ex} was unity within experimental error for ethyl toluoate, and slightly inverse for *iso*propyl toluoate arguing against proton transfer being rate limiting for exchange. The SKIE on the partitioning ratio (k_{ex}/k_{hyd}) was also shown to be approximately unity within experimental error for both esters. We therefore suggest that the O's of the tetrahedral intermediate are protonically equilibrated with the most likely mechanism for proton transfer involving a concerted shuttling of protons between the two oxygens of To^- through two bridging water molecules.

Appendix 1-2 - Supplementary Material to Part 1 - Chapter 2

Table A-1. ^{18}O Exchange of Ethyl Toluoate in 0.00981 M NaOH, T = 25°C, μ = 0.1 (NaCl).

$\%^{18}O$	time (min)
31.87	0
29.81	82
29.41	123
28.71	164
27.77	205
27.10	246

The pseudo-first order rate constant for exchange, calculated from the slope of a least squares linear regression fitting of ln (% ^{18}O) vs. time, is $k_{ex} = (1.07 \pm 0.07) \times 10^{-5}\ s^{-1}$. Errors in k_{ex} given as ±1 standard deviation in the slope calculated from ln (% ^{18}O) vs. time.

Table A-2. ^{18}O Exchange of Ethyl Toluoate in 0.00981 M NaOH, T = 25°C, μ = 0.1 (NaCl).

% ^{18}O	time (min)
31.87	0
30.61	82
29.42	123
28.57	164
28.24	205
27.50	246

$k_{ex} = (1.02 \pm 0.09) \times 10^{-5}\ s^{-1}$

Table A-3. ^{18}O Exchange of *Iso*propyl Toluoate in 0.0913 M NaOH, T = 25°C, μ = 0.1 (NaCl)

% ^{18}O	time (min)
45.44	0
41.83	19.89
39.59	39.78
37.53	59.67
35.53	79.56
31.88	119.34

$k_{ex} = (4.82 \pm 0.25) \times 10^{-5}\ s^{-1}$

Table A-4. ^{18}O Exchange of *Iso*propyl Toluoate in 0.092 M NaOH, T = 25°C, μ = 0.1 (NaCl)

% ^{18}O	time (min)
43.37	0
41.05	20
38.76	40
36.81	60
34.92	80
33.64	99

$k_{ex} = (4.33 \pm 0.15) \times 10^{-5}\ s^{-1}$

Table A-5. ^{18}O Exchange of Ethyl Toluoate in 0.00927 M NaOH, T = 25°C, μ = 0.1 (NaCl).

% ^{18}O	time (min)
30.48	0
29.62	41
29.50	82
28.60	123
28.15	164
27.13	205

$k_{ex} = (8.9 \pm 1.1) \times 10^{-6}\ s^{-1}$

Table A-6. ^{18}O Exchange of *Iso*propyl Toluoate in 0.0922 M NaOD, T = 25°C, μ = 0.1 (NaCl)

% ^{18}O	time (min)
43.29±0.30	0
40.71±0.40	20
39.25±0.28	30
35.86±0.24	40
38.16±0.35	60
33.54±0.55	80
31.34±1.2	100

$k_{ex} = (5.37 \pm 0.07) \times 10^{-5}\ s^{-1}$

Table A-7. ^{18}O Exchange of Ethyl Toluoate in 0.00989 M NaOD, T = 25°C, μ = 0.1 (NaCl)

% ^{18}O	time (min)
30.57	0
29.88	40
29.40	60
29.09	80
28.78	100
28.33	120
27.65	165
26.88	205

$k_{ex} = (1.04 \pm 0.02) \times 10^{-5}\ s^{-1}$

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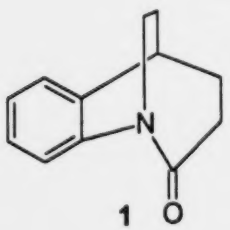
Part 2

An Investigation of the Reactivity of Bifunctional Thiol Carboxylic Acids Towards a Distorted Amide*

Introduction

The second project which will be described in this thesis involves the study of a series of molecules, **2-6,** as potential catalysts for the hydrolysis of distorted amide **1**.

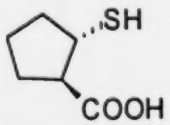


1

$HS\text{-}CH_2\text{-}COOH$ **2**

$H_3N^+\text{-}CH_2\text{-}COOH$ **3**

$HS\text{-}CH_2\text{-}COOEt$ **4**



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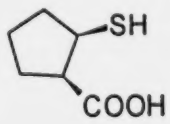

6

This is part of a continuing project in this group to investigate the mechanisms of catalysis of cleavage of the amide bond with particular emphasis on the investigation of small molecule bifunctional catalysts that can effect cleavage under mild conditions of neutral pH and room temperature. A detailed understanding of the chemistry of simple systems such as those studied here should provide some insight into the mechanisms by which the most efficient catalysts known, the enzymes, accomplish their tremendous rate

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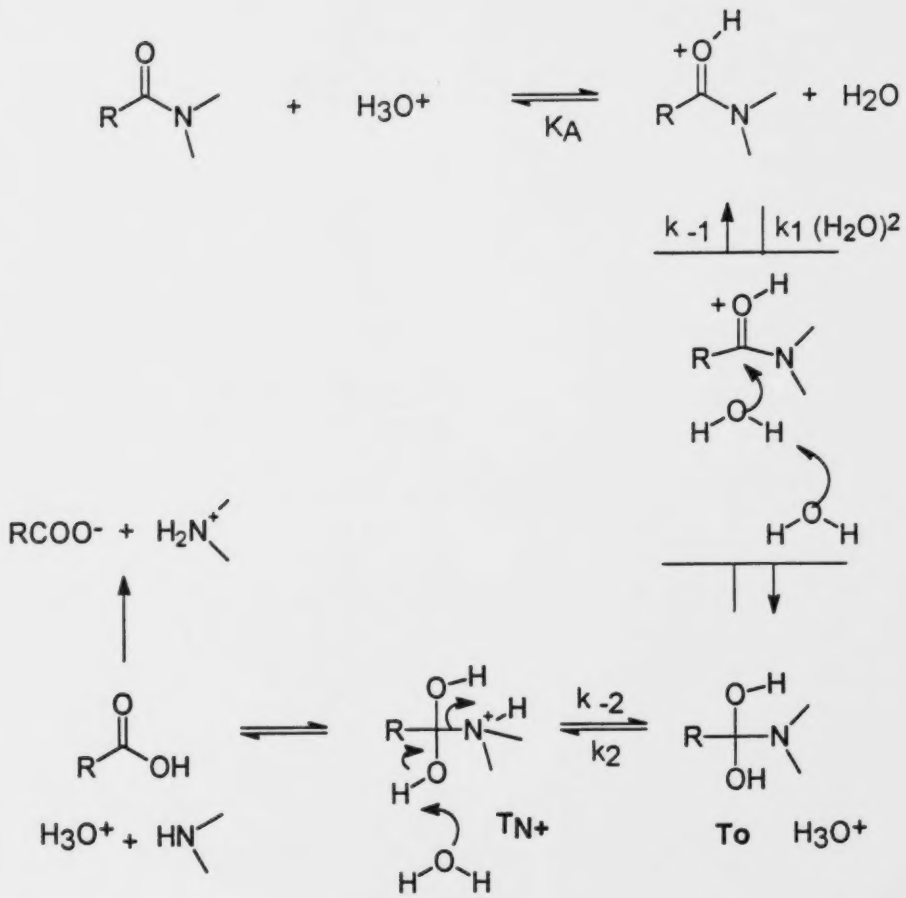
* A version of this chapter will be submitted for publication in *J. Am. Chem. Soc.*

accelerations. However, apart from any applicability to the understanding of enzymatic catalysis, the study of these small molecule catalytic systems is of importance simply because it provides the information required to formulate general principles of catalysis in aqueous solution.

(A) Mechanisms of Amide Bond Cleavage in Acidic and Basic Solution. The catalysis of the hydrolysis of the amide bond has been actively investigated for over forty years now. The sustained interest in this subject is primarily due to the fundamental importance of acyl transfer reactions involving amides in biological systems. As a result of this intensive study the mechanisms of hydronium ion and hydroxide ion catalyzed cleavage of the amide bond are now well understood.[1] A currently accepted mechanism for hydronium ion catalysis is given in **Scheme 1**[1a, 2]. As shown, the mechanism involves pre-equilibrium protonation of the carbonyl O[3] followed by general base catalyzed attack of H_2O by a second H_2O molecule leading to the neutral tetrahedral intermediate To plus H_3O^+. This tetrahedral intermediate then breaks down, following N protonation, with general base assistance from a water molecule. The rate limiting step in the acid catalyzed hydrolysis of amides depends on the relative barriers for C-N vs. C-O cleavage. Because a proton must be installed on N prior to (or concurrent with) C-N cleavage, the major determinant of the relative sizes of k_{-1} and k_2 will be the amine basicity. For regular amides having amine portions of relatively high basicity (pK_A ($^+NH_2R_2$) ≈ 10-13) N protonation will be fast and k_1 (attack of H_2O on protonated amide) will be the rate limiting step.

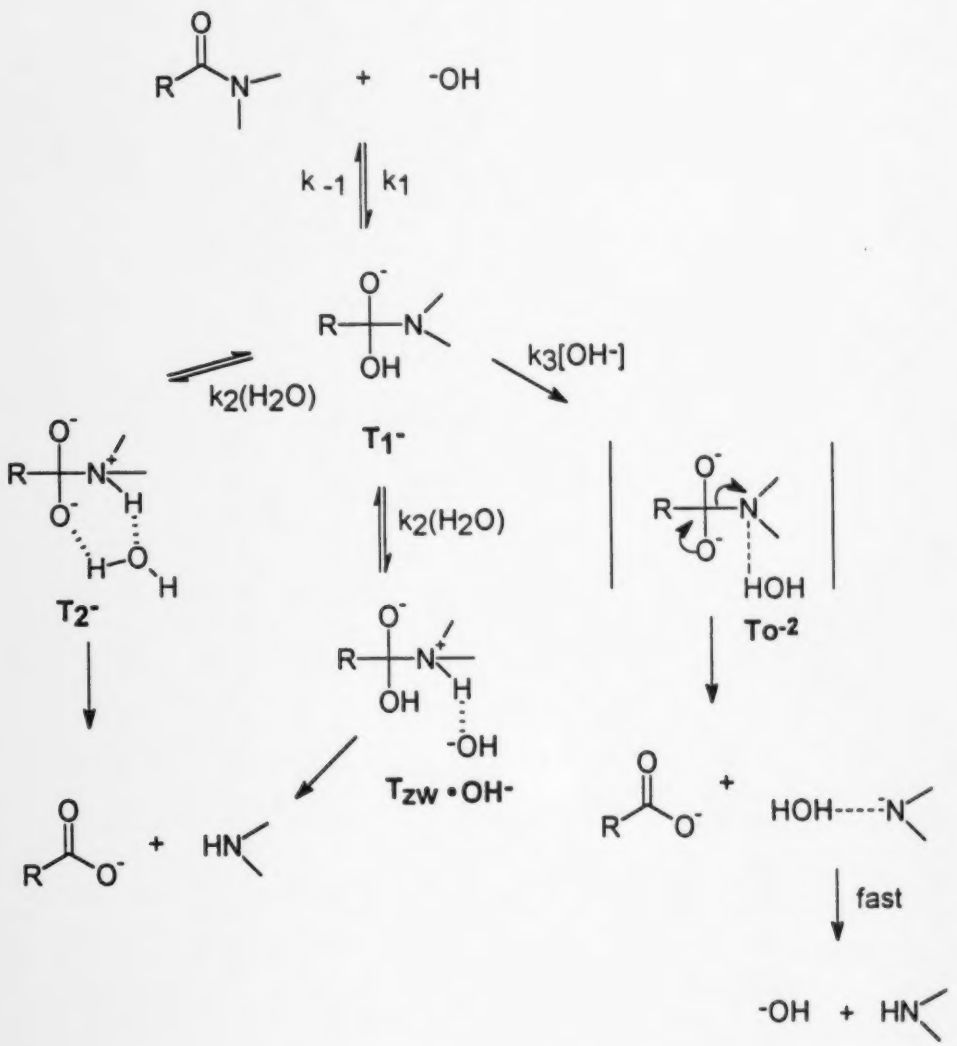
Scheme 1:



The mechanism for the hydrolysis of amides in base is given in **Scheme 2** and involves attack of OH^- on amide to form the anionic tetrahedral intermediate, T_1^-, which may then breakdown to products via an uncatalyzed (i.e. H_2O catalyzed) pathway (k_2), or through an OH^- ion catalyzed pathway (k_3) involving a dianionic tetrahedral intermediate or transition state. The k_3 pathway, involving deprotonation of T_1^- by a second OH^- followed by expulsion of amide ion ($^-NR_1R_2$), is only important in amides containing amine moieties of very low basicity (pK_A ($^+NH_2R_2$) < 3-4). For most regular amides having

amine portions of high basicity, breakdown of T_1^- occurs exclusively through k_2. For this route as in the case of acid catalyzed hydrolysis, the key factor in determining which step is rate limiting will be the amine basicity.

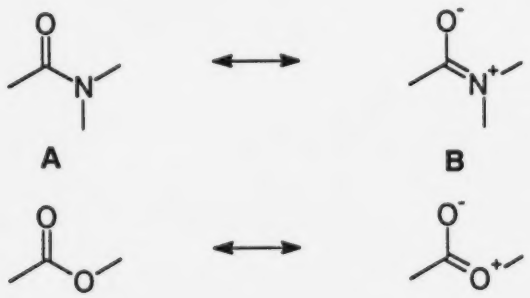
Scheme 2:



If the amine N is easily protonated (as for example in N,N-dialkyl amides) then k_1 will be the rate limiting step. If, as in the case of 2° N-alkyl amides, the amine N basicity is

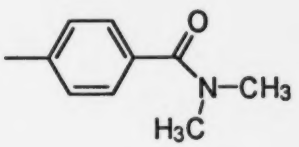
slightly lower, then both steps (formation of T_1^-, k_1, and breakdown of T_1^-, k_2) will be partially rate limiting. Further reductions in the basicity of the amine will lead to rate limiting breakdown. From SKIE studies, it appears that in the water catalyzed breakdown step (k_2), the proton is completely installed on the N prior to C-N cleavage. The proton donor may be a H_2O molecule in which case the intermediate (T_{zw}•OH^-) is formed, or a H_2O molecule may act as a shuttle removing a H^+ from the OH group of the tetrahedral intermediate and installing it on N to form the intermediate T_2^-. Decompostion of either of these intermediates leads to the formation of carboxylate and amine.

Amides are known to be much less suceptible to nucleophilic attack than are esters. This reduced reactivity has been attributed to the smaller -I effect of N vs. O, making resonance structure **B**, a more important contributor for amides than for esters.

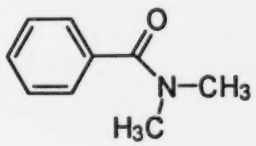


(However see Wiberg and others for a challenge to this explanation.[4]) As a result of this, amides hydrolyze more slowly than esters in base and forcing conditions are usually required for their hydrolysis. (For example $k_{OH^-}(CH_3C(O)NH_2)/k_{OH^-}(CH_3C(O)OEt) = 0.04$, H_2O, 25°C[5], where k_{OH^-} is the apparent second order rate constant for reaction with OH^-.) However amides are also much stronger bases than esters (pK_A's of protonated amides[5] range from -1 to -3, while those of protonated esters[6] usually range from -6 to -8).

Because of this, amides are more extensively protonated in acidic solution than esters and so hydrolyze much faster. For example, k_{H+} ($CH_3C(O)NH_2$) / k_{H+} ($CH_3C(O)OEt$) = 1280, H_2O, 25°C[5] (where k_H^+ is the apparent second order rate constant for hydrolysis in acid). The rate constants and half times for hydrolysis of some typical nonactivated benzamides in acidic and basic solution are given below[7a,b]:



T = 100°C, μ = 1.0 (KCl)
k_{OH-} = 1.02x10^{-3} M^{-1}s^{-1}
$t_{1/2}$ (1M NaOH) = 11.5 min
About 1.3 hrs required for complete hydrolysis.



T = 100°C, μ = 1.0
k_{H+} = 1.02x10^{-4} M^{-1}s^{-1}
$t_{1/2}$ (1M HCl) = 113 min.
About 13 hrs required for complete hydrolysis.

Based on these rate constants, the half time of hydrolysis for a tertiary amide of this type at pH 7 and 100°C is calculated to be 197 years (assuming no background water reaction).

In a 1988 paper, Kahne and Still[8] were able to directly measure the rate constant for hydrolysis of the C terminal amide bond in a tetrapeptide at neutral pH and room temperature by radiolabelling the C-terminal glycine so that minute amounts of amide cleavage products could be detected. They measured a rate constant for the water reaction with the C terminal amide bond of 3x10^{-9} s^{-1}. This converts into a half time of 7.3 years for the hydrolysis of this C terminal amide bond in neutral aqueous solution at 25°C. The significant water reaction which must exist in this case (compare this half time of 7.3 years at 25°C, to that calculated above for benzamide at 100°C) might be attributed to the

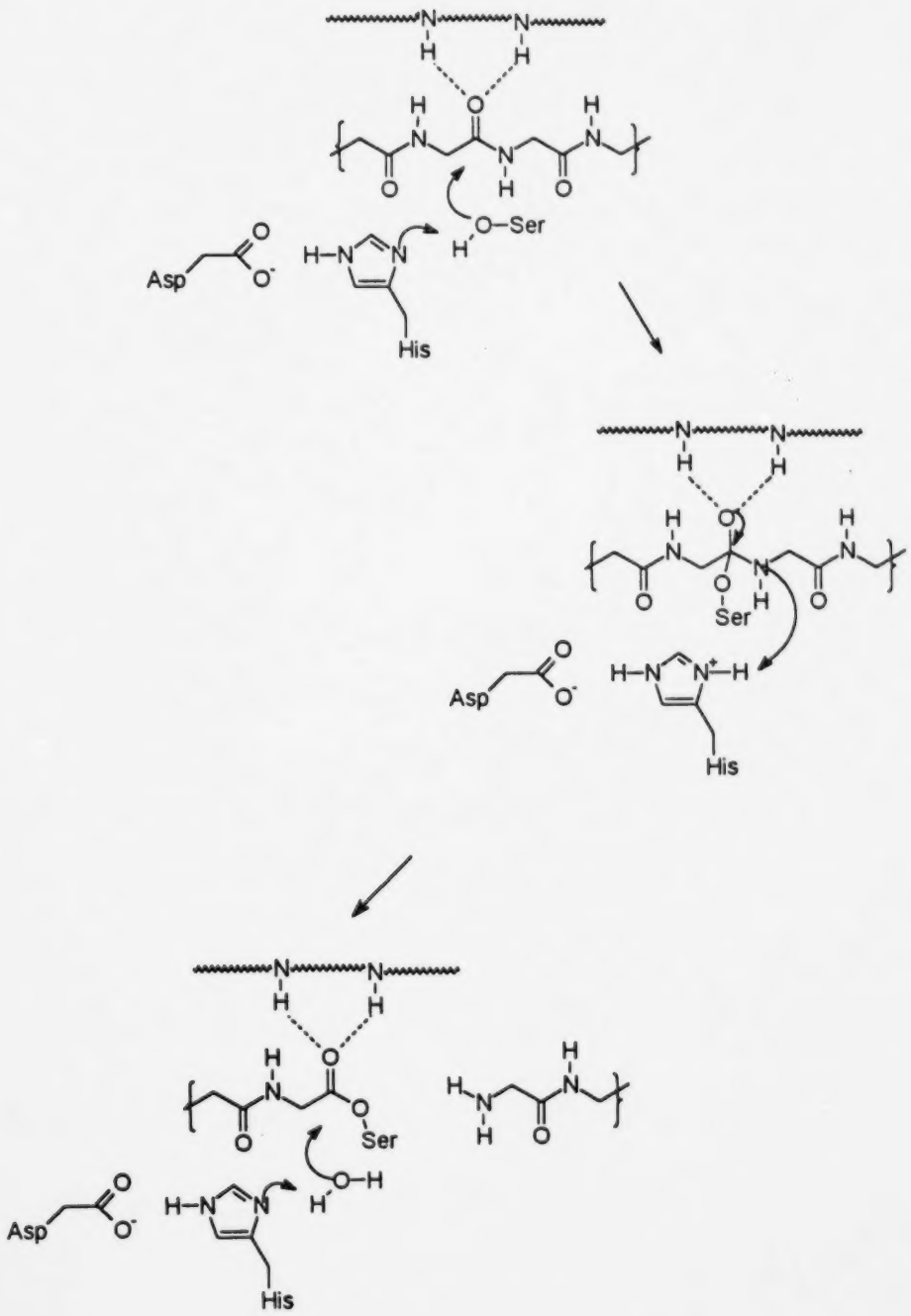
assisted delivery of H_2O to the C terminal amide bond by the α-carboxylate[9] although a noncatalyzed spontaneous water reaction cannot be ruled out.

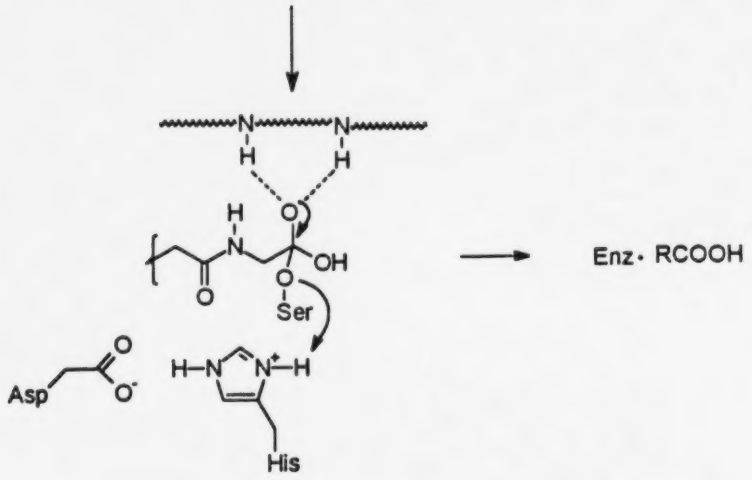
(B) Enzymatic Processes. The above demonstration of the great stability of amides in aqueous solution makes the process of enzymatic cleavage at neutral pH and 25°C seem even more remarkable. For example, a good amide substrate bound to the enzyme Carboxypeptidase A will react with H_2O (at pH 7, 25°C) with a rate constant[10] of 120 s^{-1}. This translates into a half time of hydrolysis of 0.006 seconds !

How do enzymes accomplish these fantastic rate accelerations? Direct mechanistic studies on enzymes coupled with chemical model studies have now provided much information on this question. It appears that there are 4 major classes of enzymes that cleave peptide (amide) bonds. These are the serine, cysteine, aspartate, and Zn^{+2} proteases. Each of these classes uses a unique set of catalytic machinery to cleave the amide bond. The currently accepted mechanisms for these 4 classes of protease enzymes are given below.

Serine proteases utilize 3 catalytically important groups in the cleavage of peptide bonds; a serine, a histidine and an aspartate residue as shown in **Scheme 3** below. In this mechanism the ionized aspartate residue appears to act as an 'electrostatic anchor' which holds the imidazoyl group in position[11].

Scheme 3:

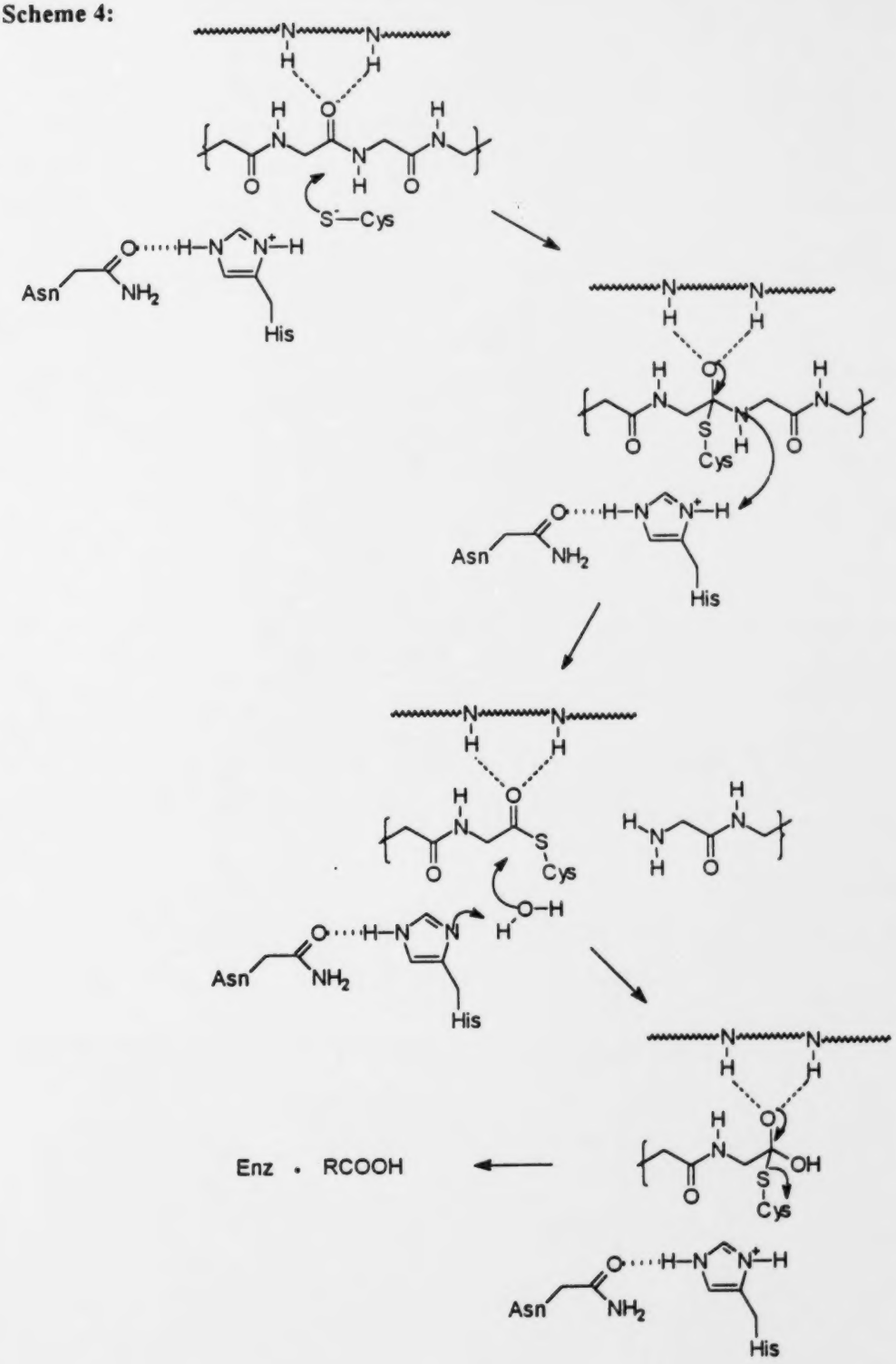




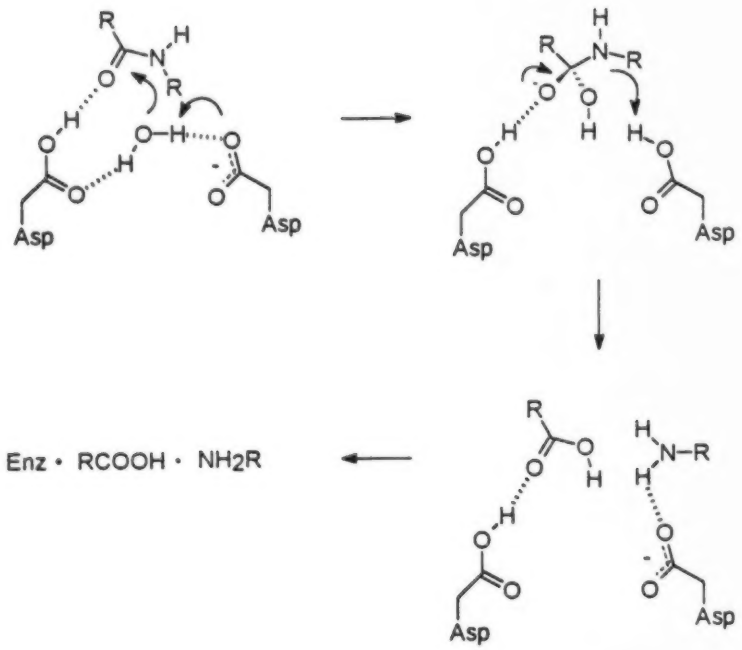
The Cysteine proteases utilize a zwitterionic imidazolium-thiolate ion pair to achieve catalysis[12] as shown in **Scheme 4.** The final expulsion of Cys-S^- from the second tetrahedral intermediate is uncatalyzed in contrast to the general acid catalyzed expulsion of Ser-O^- in the Serine protease mechanism, which is in accord with the much better leaving group ability of Cys-S^- vs. Ser-O^-.

The aspartate proteases carry out peptide bond cleavage using 2 catalytically active aspartate residues.The mechanism is still controversial due to the question of whether the mechanism involves nucleophilic attack of an aspartate carboxylate to form an acyl enzyme intermediate or whether the mechanism involves an aspartate residue initially acting as a general base for the attack of water. However current opinion tends to favour general base/general acid catalysis by one of the active site aspartyl residues with the other protonated residue acting to stabilize the developing negative charge on the C=O oxygen by a hydrogen bonding interaction[13]. This mechanism is shown in **Scheme 5.**

Scheme 4:



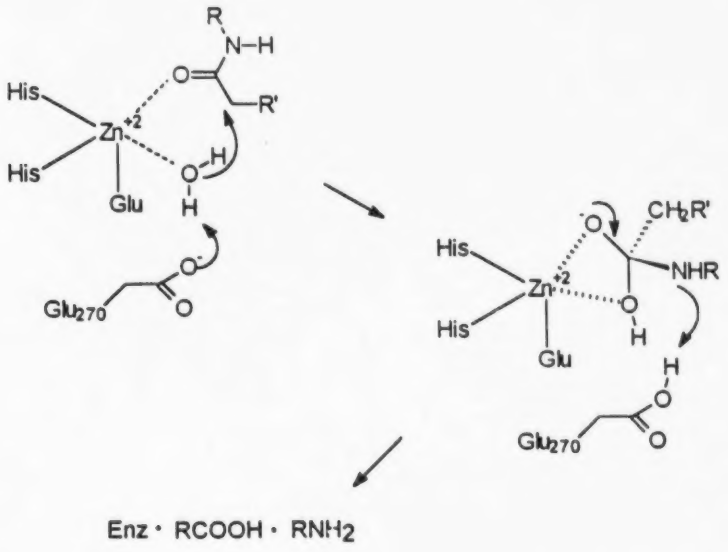
Scheme 5:



The active site of zinc proteases of which Carboxypeptidase A is the most well studied example, contain a Zn^{+2} ion ligated by 2 histidine imidazoles and a glutamate residue. A fourth coordination site appears to be occupied by a water molecule. Also found in the active site are a conserved glutamate (Glu 270) and tyrosine residue (Tyr 248), which seem to be necessary for catalytic activity. Many proposals have been put forward with regard to the catalytic mechanism of this enzyme[14]; the one currently favoured[15] involves Glu 270 acting as a general base on the zinc bound water molecule as it attacks the carbonyl of the scissile amide bond, which may also be coordinated to the

Zn^{+2} center. This mechanism is shown in **Scheme 6**. (For a recently proposed alternative mechanism involving the substrate carboxylate acting as general base see Mock and Xu[16].)

Scheme 6:



Enz · RCOOH · RNH_2

The function of the conserved tyrosine residue is unknown although its initially postulated role as a general acid catalyst for protonation of N in the tetrahedral intermediate[17] has been shown to be incorrect from a site directed mutagenesis study that replaced Tyr 248 with a Phe residue.[18]

In summary, the methods of catalysis that enzymes use to cleave amide bonds appear to include the following: 1). Electrophilic catalysis of attack of a nucleophile on the amide C=O, either via coordination of the carbonyl O to a metal center, or by H bonding of the carbonyl O to donor groups in the enzyme (Zn^{+2}, Ser, Cys and Asp proteases). 2). General base catalysis of nucleophilic attack of ROH or H_2O by imidazole (Ser proteases). 3). General base catalysis of attack of H_2O by a carboxylate (Asp, Zn^{+2} proteases). 4).

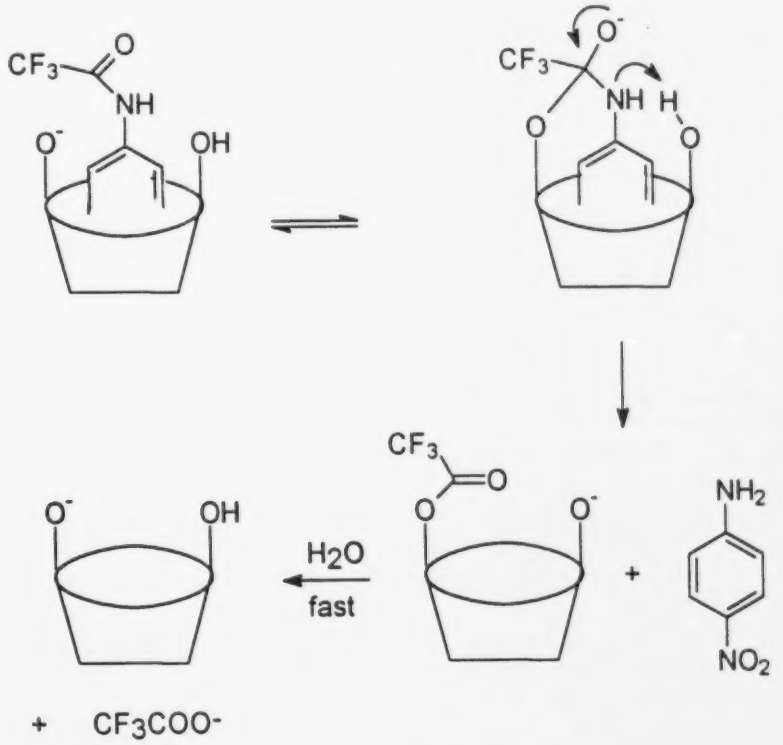
Nucleophilic catalysis by attack of thiolate on the amide carbonyl (Cys proteases). 5). General acid catalysis of the departure of N from the tetrahedral intermediate by imidazolium ion or a protonated carboxyl group (Ser, Cys, Asp, and Zn^{+2} proteases). However, probably the most important single factor responsible for the large rate accelerations seen in all of these enzymes is the use of the free energy resulting from binding of substrate to enzyme, to hold one or more reacting groups in close proximity to the substrate so that reaction can occur rapidly (Proximity effect).[19] Due to this fact it will probably be extremely difficult to construct a synthetic catalyst that will be able to match the efficiency of an enzyme, however much important information on catalytic processes can still be gained from such synthetic endeavors. Some examples of attempts to make synthetic catalysts for amide hydrolysis are described in the following paragraphs.

(C) Small Molecule Catalytic Systems. Most examples to date of cleavage of amide bonds under mild conditions have been intramolecular in nature. These systems are stoichiometric, not catalytic, since the catalytic and the amide functionalities are present in the same molecule. Although much information has been gained from such studies[14] on the nature and magnitudes of the factors responsible for enzymatic rate accelerations, a more challenging goal is to construct true catalysts which react in a bimolecular fashion with an amide substrate and are released unchanged after the cleavage event. Some initial progress has been made in this area as discussed below.

In a 1977 paper[20] Bender and Komiyama showed that α-cyclodextrin (α-CD) was able to catalyze the cleavage of *p*-nitrotrifluoroacetanilide at near neutral pH's and 30°C. They found that the rate of cleavage of α-CD bound *p*-nitrotriflouroacetanilide was 16-20

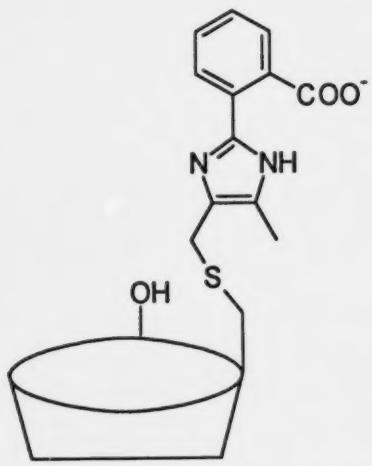
times faster than the rate of background cleavage of the amide at pH 6-7. Studies done using α-CD concentrations less than that of amide showed that the reaction was still pseudo first order indicating that α-CD is not used up during the reaction and that true turnover must be occurring. A plot of log k_c (first order rate constant for cleavage of amide bound to α-CD) vs. pH showed a plateauing above pH 12.1 corresponding to the pK_A of the titratable 2° OH of α-CD. Based on this data a mechanism was proposed **(Scheme 7)** involving attack of an ionized 2° OH group of α-CD on bound amide.

Scheme 7:



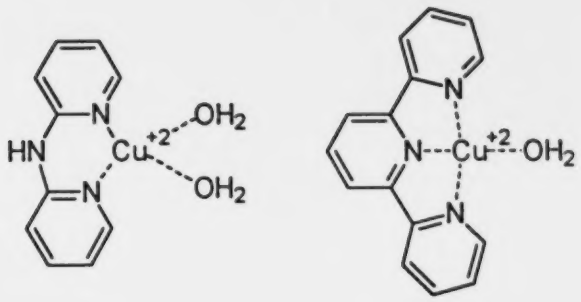
The observation of a large normal SKIE on the catalyzed reaction is in accordance with the proposed general acid catalyzed breakdown of the tetrahedral intermediate by a neighbouring unionized hydroxyl group of α-CD.

Bender and coworkers also synthesized a miniature organic model of chymotrypsin, **7**, consisting of a β-cyclodextrin binding site with attached hydroxyl, imidazoyl and carboxylate functionalities[21]. It was claimed that this enzyme model cleaved the ester substrate *m-tert*-butyl phenylacetate faster than chymotrypsin cleaves pNPA. However, no description of the catalytic activity of this model enzyme towards an amide substrate was given. It was later shown[22] that the 'catalytic triad' present in this model does not actually participate in the hydrolysis of ester substrates (in fact the carboxy-phenyl imidazoyl moiety actually retards the binding and hydrolysis of ester substrates by the cyclodextrin).



7

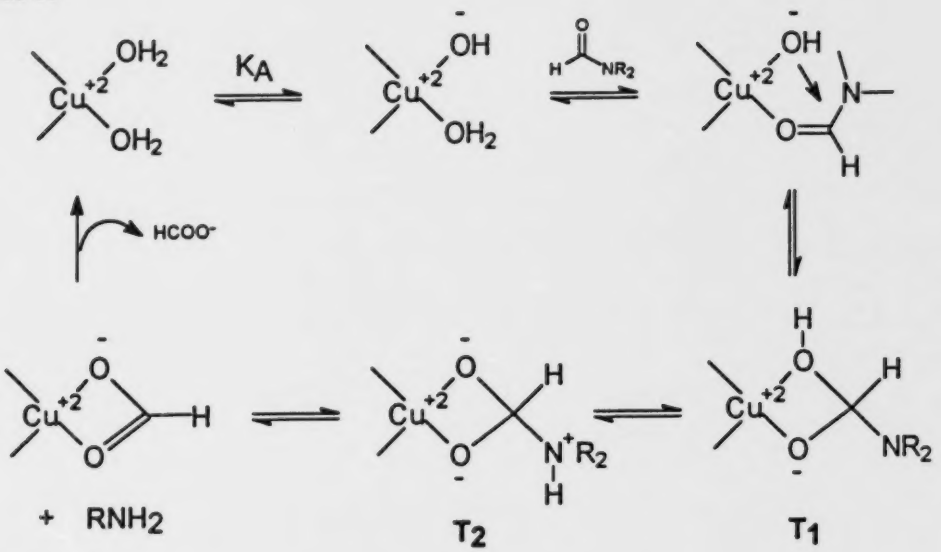
Chin and coworkers have demonstrated that the diaquocopper (II) complex **8**, can act as a true turnover catalyst in the hydrolysis of unactivated esters and amides[23].



8 **9**

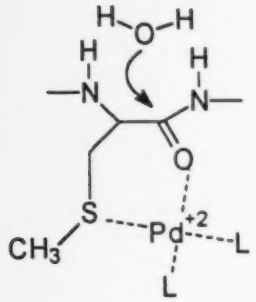
The hydrolysis of MeOAc could be catalyzed at 25°C, with a maximal value of k_2^{obs} of 7.2×10^{-4} $M^{-1}s^{-1}$ being observed at pH's > 7.2 corresponding to the pK_A of the metal bound H_2O. This indicates that the aquo-hydroxo metal complex is the active form of the catalyst. Metal complex **8** is also able to catalyze the hydrolysis of a series of unactivated formamides at 100°C. A maximal 2nd order rate constant of 1.9×10^{-3} $M^{-1}s^{-1}$ was observed for the hydrolysis of formamide above pH 7.2. Monoaquo complex **9** did not catalyze the hydrolysis of either unactivated esters or amides to any observable extent. The mechanism proposed for amide hydrolysis involves coordination of the amide C=O to the copper center followed by intramolecular metal hydroxide attack as shown in **Scheme 8**. It is proposed that the copper center activates the C=O, provides a template for intramolecular attack of ^{-}OH on amide, and stabilizes the normally high energy tetrahedral intermediate T_2 which is required for expulsion of the amino group.

Scheme 8:

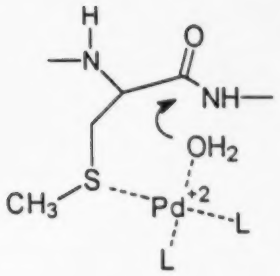


Brown and coworkers[24] have also developed a catalyst capable of catalyzing the hydrolysis of unactivated formamides in true turnover fashion (100°C) with an efficiency similar to that of **8**. This work will be described in more detail in the next section when bifunctional catalysts are discussed.

A study involving the selective hydrolysis of unactivated peptide bonds adjacent to cysteine and methionine residues by Pd^{+2} and Pt^{+2} complexes was carried out by Kostic *et al.*[25]. These workers found that various diaquopalladium (II) complexes could catalyze hydrolysis of peptide bonds at pH 2 and 40°C by coordinating to various sulfur centers in the peptide. The reaction may involve either displacement of a H_2O molecule at the metal center by the amide C=O, followed by intermolecular attack of H_2O as in **A** or the intramolecular delivery of a metal bound H_2O molecule to the C=O of the scissile amide bond as in **B**.



A

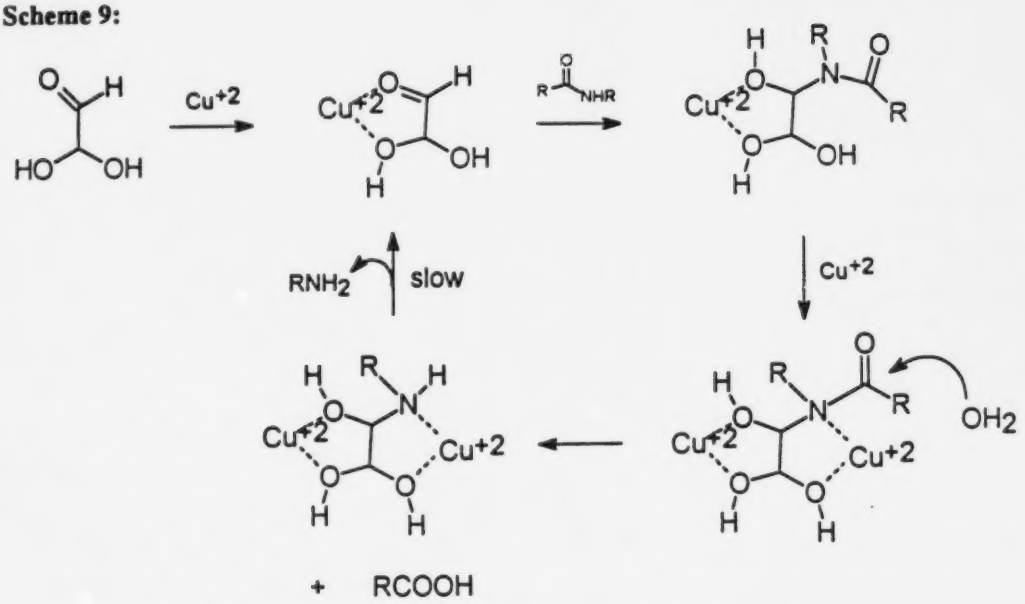


B

These reactions are kinetically indistinguishable. The reaction has to be carried out at pH ~ 2 since above this pH bridged palladium-hydroxo compounds are formed which precipitate from the solution. The regiospecific cleavage of peptide bonds described in these studies is stoichiometric and does not involve catalytic turnover of the Pd^{+2} complexes.

One final example involves the development of a system by Singh and Ram[26] to selectively hydrolyze 1° and 2° amides in the presence of esters under somewhat mild conditions (refluxing water / 30% glyoxal, 8-25 hrs.). In this method an additional metal ligating group is created at the nitrogen of the amide substrate in situ, by taking advantage of the reactivity of the N-H bond towards aldehydes. The details of this process are shown in **Scheme 9**. Upon reaction of the amido N with the copper bound glyoxal, the N and the glyoxilic hydroxyl group become ideally positioned for bidentate chelation to a second copper center, thus activating the amide towards nucleophilic attack and also activating the leaving group.

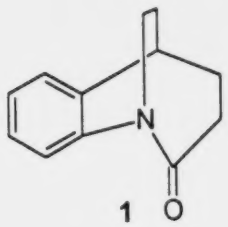
Scheme 9:



The authors claim that reaction will take place when only a 'catalytic' amount of Cu^{+2} is used (20 mol %) indicating that turnover will occur. However reaction times are about twice as long.

From this discussion of the studies that have been carried out to date on the catalysed hydrolysis of amides in aqueous solution, it is evident that the goal of constructing a catalyst that will cleave unactivated amide bonds under mild conditions of temperature and pH has so far proven elusive.

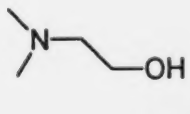
(D) Studies of the Reactions of Bifunctional Catalysts with a Distorted Amide. A large amount of work directed at the study of the reaction of bifunctional molecules with amide **1** has been carried out by Brown and coworkers since 1985.



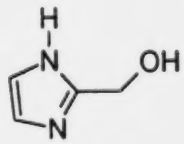
1

Amide **1** is activated in the sense that there is an enforced distortion away from planarity of the amide bond.[27] However this molecule is still a better substrate for the study of hydrolytic reactions involving amides than the popular ester substrate pNPA since it should have the same catalytic requirements for hydrolysis as a nonactivated amide, subsequent to the initial nucleophilic attack to form the tetrahedral intermediate.

In an initial study[28], β-amino alcohols **10-12** were investigated for their ability to catalyze the ring opening of **1**.



10



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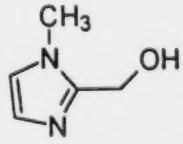
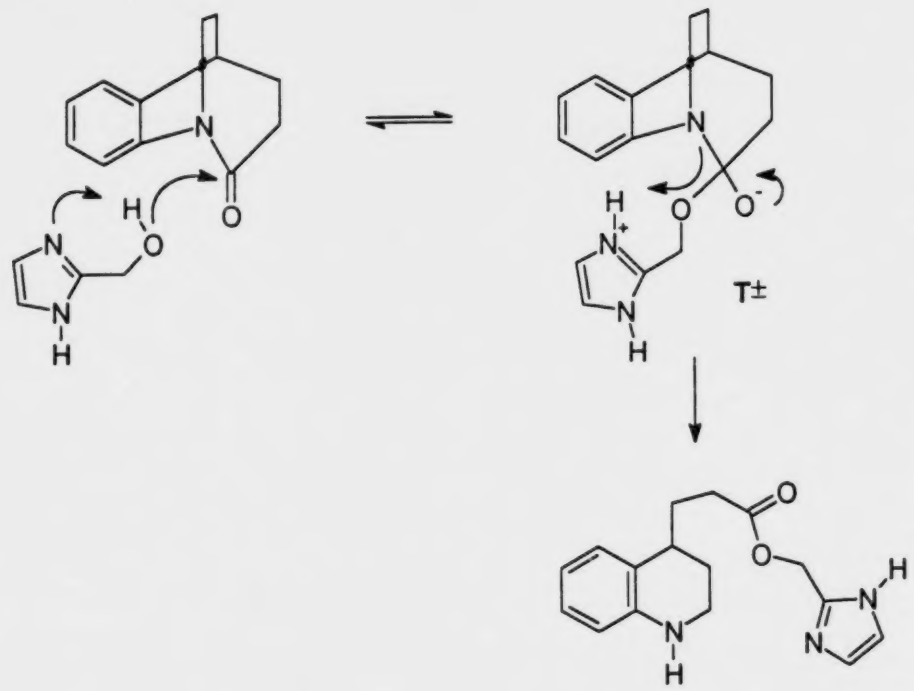


12

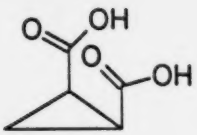
These amino alcohols all exhibited the same type of pH-rate profile in their reaction with **1** indicating that the basic form of the amino alcohol was active. In all cases the β-amino alcohols were found to be more reactive towards **1** than were the corresponding amines lacking the hydroxyl group. This, coupled with the fact that product studies showed that the oxygen ester was the major product of the reaction, led to the proposal of the mechanism shown in **Scheme 10**.

Scheme 10:

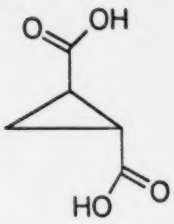


This mechanism involves intramolecular general base catalyzed attack of the OH group on the amide C=O to form tetrahedral intermediate $T^{\pm}$, whose breakdown is further facilitated by intramolecular general acid catalysis by the protonated N of the pendant amine. This intramolecular general base catalyzed acyl transfer reaction can be considered as a model for the acylation step in serine proteases.

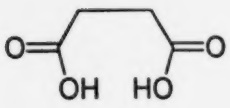
Amide **1** was also shown to be highly susceptible towards attack by a series of dicarboxylic acids capable of forming cyclic anhydrides[29]. Dicarboxylic acids **13-15** were found to be much more efficient in promoting the decomposition of **1** than were dicarboxylic acids **16** and **17** which cannot form cyclic anhydrides easily.



13



16



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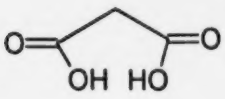
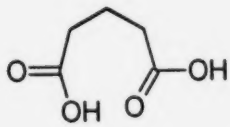


17



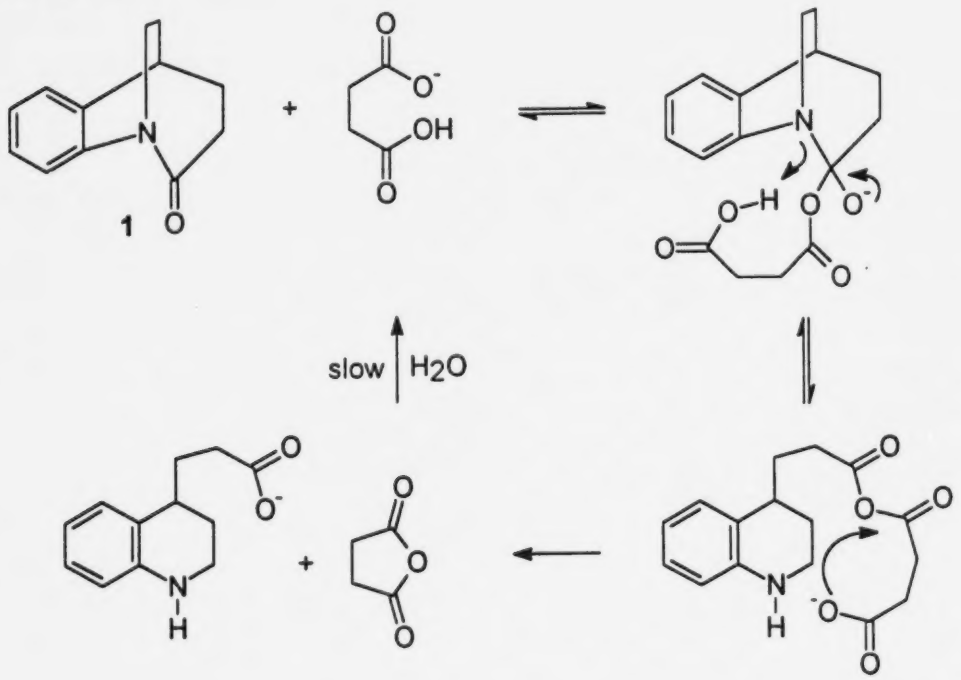
15

IR studies carried out in CH_3CN showed that ring opening of **1** in the presence of **13**, **14** or **15** was accompanied by the formation of a cyclic anhydride product. Based on this evidence, and on the observed pH rate profile, a mechanism was proposed for the higher pH regions involving nucleophilic attack of the monoanionic form of the dicarboxylic acid on the amide, leading to the transient formation of an open anhydride. This is rapidly captured by internal cyclization to form a cyclic anhydride and amino acid product (**Scheme 11**). The role for the pendant -COOH as a general acid catalyst for the breakdown of the tetrahedral intermediate is supported by the observation that the monoanion is more reactive towards amide **1** than the dianion.

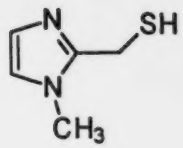
The mode of reaction of these dicarboxylic acids with **1** has relevance to the mechanism of action of aspartate proteinases since a nucleophilic mechanism involving the active site aspartyl groups was at one time a favoured mechanism. However since no anhydride intermediates could ever be detected[30], this mechanism has been superseded by

one involving general acid / general base catalysis (see **Scheme 5**). It is of note however, that the anhydride intermediates assumed to be formed during the reaction of **13-15** with **1** were also not detectable in aqueous solution.

Scheme 11:



In a third study[31], the reactivity of a series of ammonium thiolates towards amide **1** were investigated as a model of the acylation step of cysteine proteases. The reaction of 2-(mercaptomethyl)-N-methylimidazole **(18)** with **1** was shown to follow a bell shaped pH-rate profile (log k_2^{obs} vs. pH) with a maximal second order rate constant for ring opening of **1** of 99 $M^{-1}s^{-1}$ (T = 25°C, μ = 1.0).

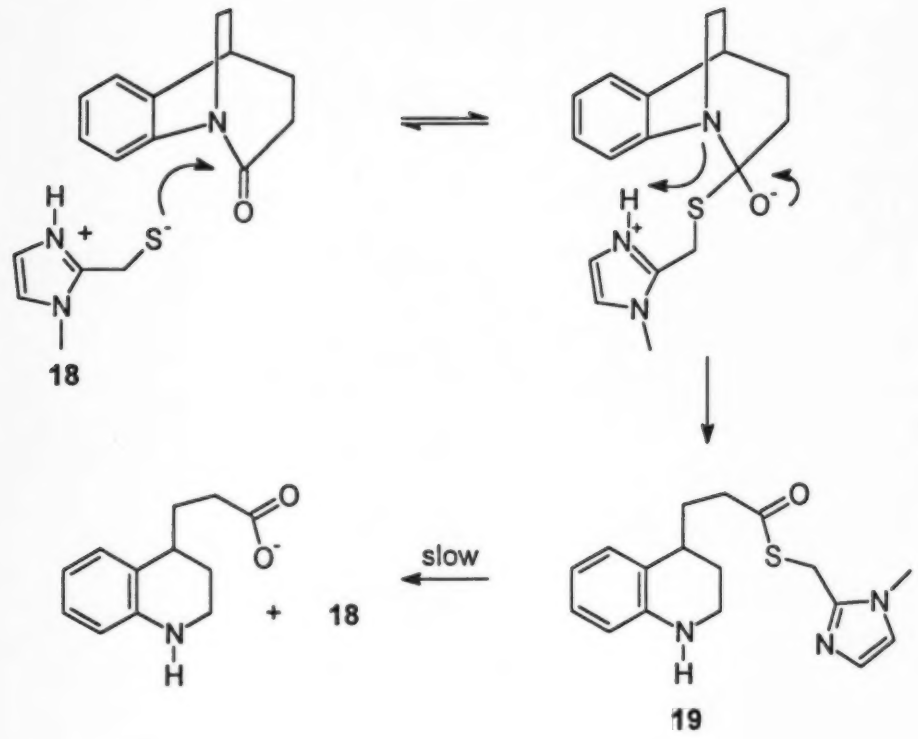


18

Since it was known that **18** exists largely in the zwitterionic ammonium thiolate form at neutral pH's[32], a mechanism was postulated involving initial nucleophilic attack of thiolate on amide, followed by general acid catalyzed breakdown of the tetrahedral intermediate by the pendant imidazolium group **(Scheme 12)**. According to this mechanism both a nucleophilic thiolate and a proton source are required for maximal catalytic activity. This conclusion is supported by control studies which show that model compounds containing only an amino or thiol functionality are relatively inactive.

It was further shown[33] that the thiolester product **19**, does hydrolyze to the amino acid + **18** with assisted general base catalysis by the pendant imidazole, but this reaction is slow compared to the acylation step (k_{obs} is ~ 1×10^{-4} s^{-1} at pH 7). However hydrolysis of **19** is still ~10× faster than hydrolysis of **1** at pH 7 so that **18** may be considered as a true turnover catalyst (if not a very efficient one).

Scheme 12:



Imidazole-thiol **18**, was also found to catalyze the hydrolysis of some nonactivated formamides at pD 7.6-8.0 and 100°C[24]. The mechanism by which **18** mediates the hydrolysis of these amides was expected to be the same as that shown above. However under these conditions no accumulation of thiol ester was observed by NMR, so that deacylation of catalyst **18** must be fast compared to its acylation. Although the observed catalysis is fairly inefficient[34] and requires high temperatures (k_2^{obs} for the reaction of **18** with $HC(O)NH_2$ at pD 7.8 and 100°C = 1.3×10^{-4} M^{-1} s^{-1}), it still remains as one of the only examples to date where true catalysis of the hydrolysis of a nonactivated amide has been achieved.

The project which will be presented subsequently in this thesis involves a study of the reaction of several bifunctional thiol-acids with amide **1**. The initial idea behind the project was to study some potential catalysts for the hydrolysis of **1** which contained a pair of functional groups not normally found together in the active site of a protease enzyme[35]. Following some initial screening experiments of molecules containing various pairs of functional groups, it was found that thioglycolate (**2**), was a fairly efficient catalyst of the ring opening of **1**. The investigation of the pH- rate behaviour of the reaction of this thiol acid as well as trans and cis cyclopentane thiol-acids **5** and **6** with amide **1** were carried out as will be described in the following pages. To obtain further information on the possible mode of reaction of these thiol acids with **1**, the reactions of glycine (an ammonium analog of **2**), and the ethyl ester of thioglycolic acid, **4**, with **1** were also investigated. Based on the results of these studies a tentative mechanism for the reaction of thiol-acids **2**, **5**, and **6** with **1** will be proposed which is consistent with previous mechanisms proposed for the reactions of amino-alcohols, dicarboxylic acids, and ammonium-thiolates with distorted amide **1**.

Experimental

(A) Materials and General Methods. The following compounds were obtained from commercial suppliers: sodium thioglycolate (Sigma), glycine (ICN), and ethyl-2-mercaptoacetate (Aldrich). Ethyl-2-mercaptoacetate was purified before use by column chromatography (silica, 97 $CHCl_3$/2 MeOH/1 CH_3COOH) to remove contaminating thioglycolic acid. Buffers, MES (2-[N-morpholino]ethanesulphonic acid), MOPS (3-[N-morpholino]propanesulphonic acid), HEPES (N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulphonic acid]) and CHES (2-[Cyclohexylamino]ethane sulphonic acid), were reagent grade (Sigma) and were used as supplied. Acetonitrile was dried over 3 angstrom molecular sieves and distilled from P_2O_5.

^1H NMR and ^{13}C NMR spectra were obtained using a Bruker WH-200 or a Bruker AM-400 spectrometer. Infrared spectra were recorded using a Nicolet Magna 750 FTIR spectrometer. High resolution mass spectra were obtained using an AEI-MS50 mass spectrometer and low resolution spectra using an AEI-MS12 spectrometer. All melting points were obtained using a Canlab Gallenkamp apparatus and are uncorrected.

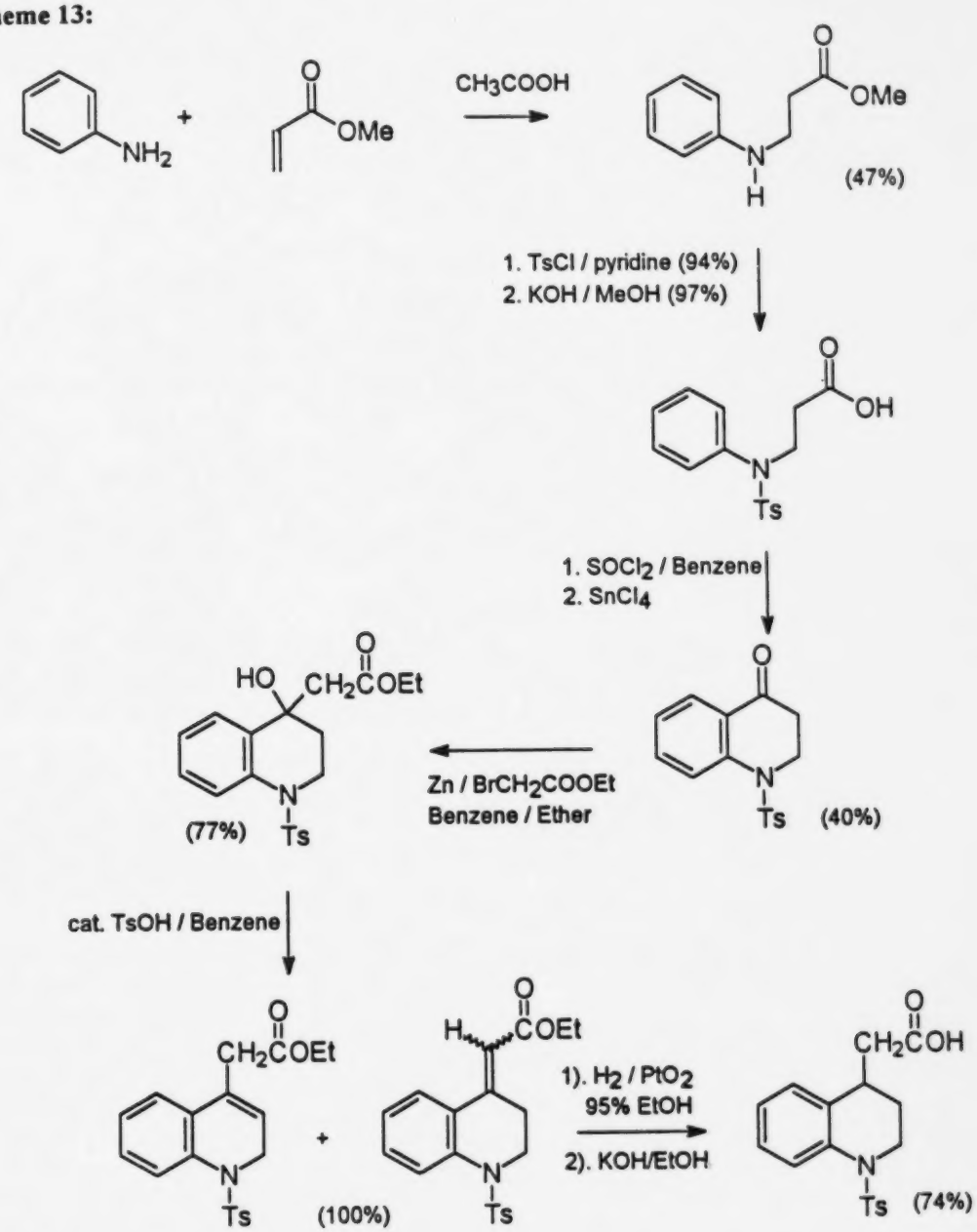
Flash chromatography was performed using silica gel 60 (Merck, 40-63 um particle size).

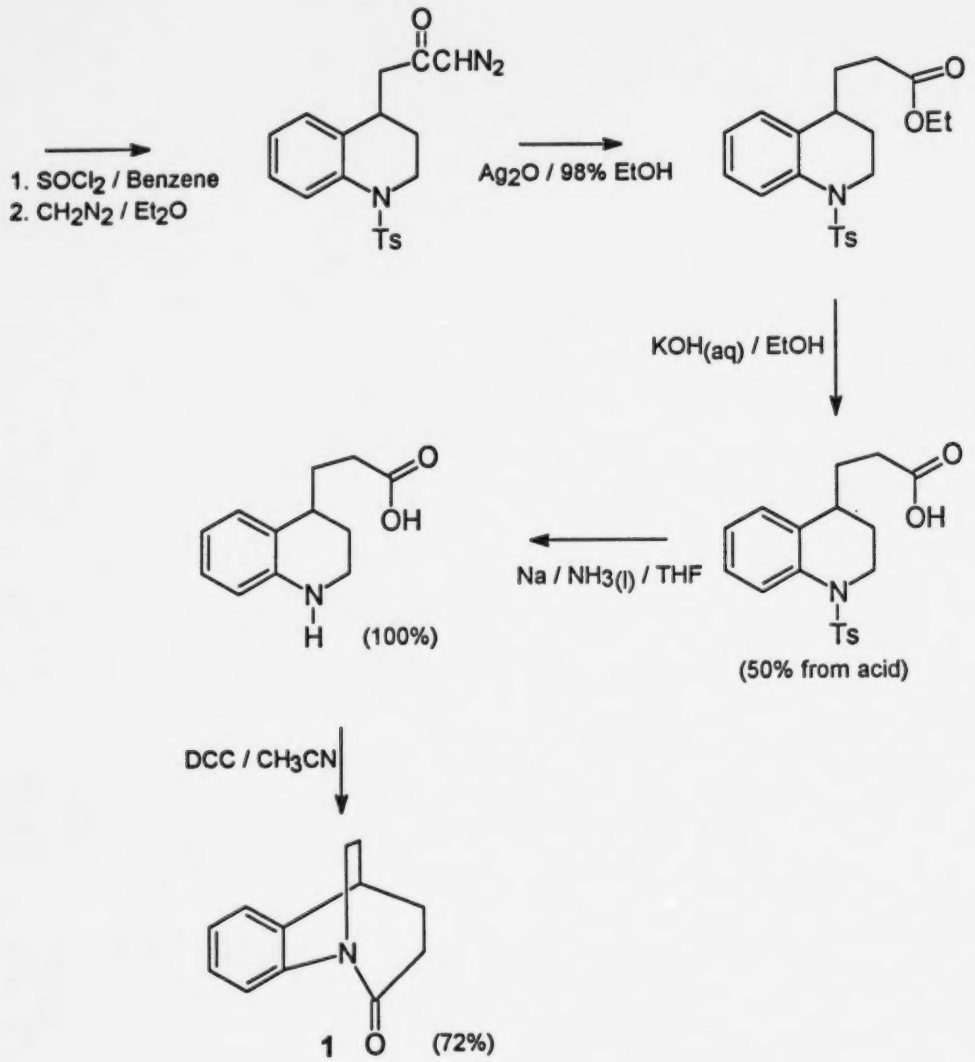
(B) Syntheses.

2,3,4,5-tetrahydro-2-oxo-1,5-Ethanobenzazepine (1): Amide **1** was synthesized from aniline and methyl acrylate using the method described previously by Brown *et al.*[36]. The 15 step synthetic route is outlined in **Scheme 13**. A 3% overall yield of purified amide

1 was obtained. All spectral characteristics for intermediates and final product agreed with literature values.

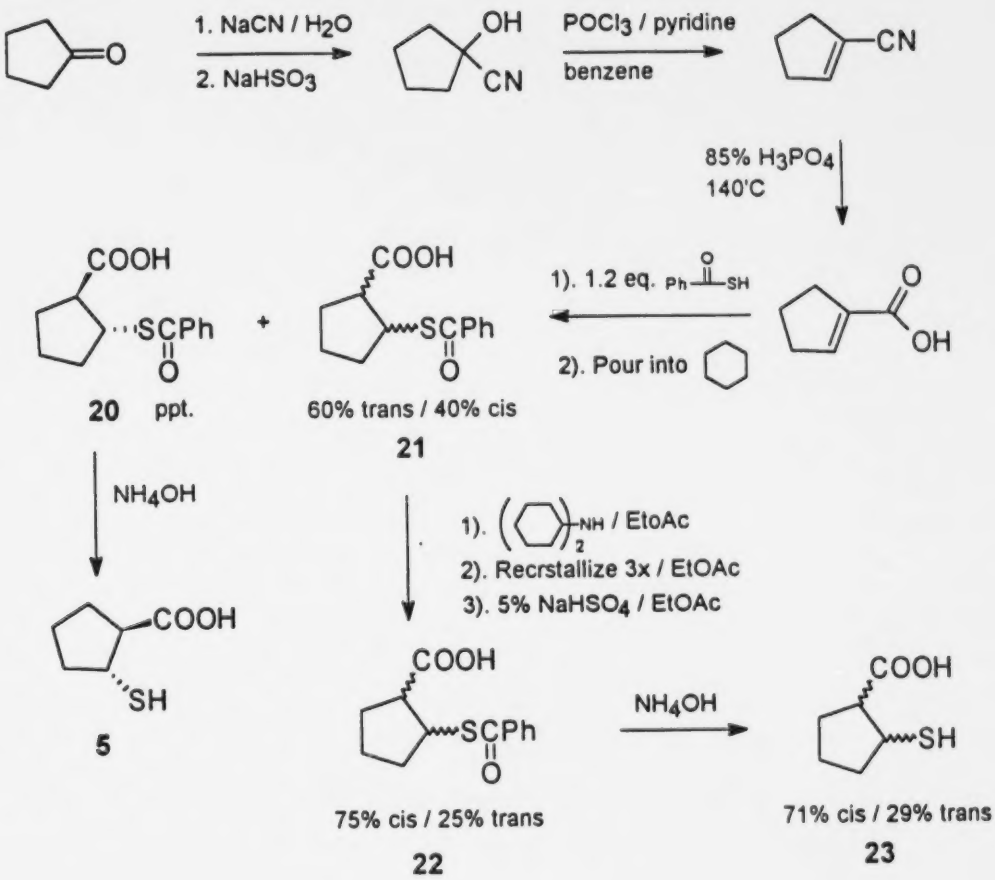
Scheme 13:





***cis* and *trans* 2-Mercaptocylclopentanecarboxylic Acids:** These compounds were synthesized according to the general method described by Ciabatti *et al.*[37]. The route followed is outlined in **Scheme 14** .

Scheme 14:



Radical addition of thiobenzoic acid to cyclopentene carboxylic acid led to a mixture of cis and trans addition products. However upon pouring the reaction mixture into cyclohexane, pure trans product **20** precipitates out, leaving a 40/60 cis/trans mixture behind in the mother liquor. The trans compound **20** was recrystallized twice from cyclohexane and twice from CH_2Cl_2 prior to removal of the S-benzoyl group by aminolysis. The cis/trans mixture was subjected to column chromatography (silica, 98 $CHCl_3$/1 MeOH/1 HCOOH) to remove excess thiobenzoic acid, followed by trituration with water (overnight stirring) to remove benzoic acid formed by decomposition of

thiobenzoic acid on the column. To allow separation of cis and trans compounds by fractional recrystallization, the mixture **21** was converted to the dicyclohexylammonium salts. A 75/25 cis/trans mixture was obtained after 3 recrystallizations. After liberation of the free acid, debenzoylation was carried out on this mixture as for the trans compound.

Spectral data for all intermediates were identical to those reported in the literature. The identification of **20** as the trans compound was confirmed by a crystal stucture (See **Appendix 2**). (Previous assignment of the trans structure to **20** had been based on chemical shifts and coupling constants of the methine protons[37] however determination of cis/trans relationships in five membered rings via NMR methods is often hazardous due to the conformational flexibility of the ring and any substituent groups[38]). The cis and trans thiol acids **5** and **23** were not synthesized by Ciabatti *et al.* so their spectral data is given below.

***trans* 2-Mercaptocyclopentanecarboxylic Acid (5):** The free thiol was obtained from **20** by aminolysis of the S-benzoyl group using NH_4OH according to the general procedure described by Ciabatti *et al.*[37]. The crude thiol acid was purified by column chromatography (silica, 96 $CHCl_3$/3 MeOH/1 HCOOH) under an Ar atmosphere using degassed solvents. The product, a white solid, was isolated in 85% yield from the thiol ester: mp 40-43°C; IR ($CHCl_3$ cast film) ν 2965, 2875, 2781, 2694, 2647, 2564, 1706, 1308 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.52-1.64 (m, 1H), 1.68-1.94 (m, 4H), 2.10-2.26 (m, 2H), 2.72 (apparent q, J = 8.5 Hz, 1H), 3.40 (apparent pentet, J = 8 Hz, 1H), 11.7 (br s, 1H); ^{13}C (75 MHz, $CDCl_3$) δ 24.13 (CH_2), 29.90 (CH_2), 36.86 (CH_2), 40.76

(CH), 55.09 (CH), 180.97 (C=O); MS (CI / NH_3) 164.1 (M^++18, 100%); Anal. calcd. for $C_6H_{10}O_2S_1$; C 49.29, H 6.89, S 21.93. Found C 49.35, H 6.83, S 21.86.

(*cis* + *trans*) 2-Mercaptocylcopentanecarboxylic Acids (23): The debenzoylation of the mixture of (cis + trans) 2- S-benzoyl cyclopentane carboxylic acids **22**, was carried out as described for the trans compound. The crude liquid was purified by chromatography (silica, 95 $CHCl_3$/3 MeOH/2 CH_3COOH) under an Ar atmosphere using degassed solvents. The product (clear liquid), a 71/29 cis/trans mixture of the thiol-acids as determined by NMR, was isolated in 97% yield from the thiol ester. This mixture was used directly in kinetic experiments without further attempts at separation. Spectral data for the mixture are given below; for the ^{13}C spectrum only those peaks corresponding to the cis compound are given. IR ($CHCl_3$ cast film) ν 2962, 2875, 2773, 2681, 2639, 2564, 1704 1307cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.53-2.01 and 2.09-2.26 (m, 7H), 2.72 (apparent q, J = 7.5 Hz, 0.29H, trans -C*H*-), 3.07 (apparent q, J = 7.5 Hz, 0.71H, cis -C*H*-), 3.42 (apparent pentet, J = 7.5 Hz, 0.29H, trans -C*H*-), 3.55 (apparent pentet, J = 7.5Hz, 0.71H, cis -C*H*-), 11.08 (br s, 1H); ^{13}C (75 MHz, $CDCl_3$) δ 22.67 (CH_2), 25.95 (CH_2), 35.85 (CH_2), 40.89 (CH), 51.28 (CH), 179.38 (C=O); HRMS (EI), exact mass calcd. for $C_6H_{10}O_2S_1$ 146.04015, found 146.04002 (77%); Anal. calcd. for $C_6H_{10}O_2S_1$: C 49.29, H 6.89, S 21.93, found: C49.30, H 6.95, S 20.17. The low analysis for S can be accounted for by an ~ 6% (by mass) impurity of acetic acid in the sample. This was confirmed by Ellman's titration which showed 94 wt. % thiol in the sample.

(C) Kinetics. The following buffers were used to control the pH; acetate (pK_A 4.76), MES (pK_A 6.1), MOPS (pK_A 7.2), phosphate (pK_A 7.2), HEPES (pK_A 7.5), CHES

(pK_A 9.3). For studies involving the reaction of thioglycolic acid with amide **1** at pH's near 4 and 10, thioglycolic acid itself was used as the buffer (pK_1 = 3.55, pK_2 = 10.22[39]). Buffers were made using purified, deoxygenated H_2O from an Osmonics Aries water purification system ($[buffer]_T$ = 50 or 200 mM, μ = 1.0 (KCl)). The buffer solutions were further degassed by bubbling Ar through them for several hours before using.

The rate of ring opening of the strained amide **1** in the presence of excess thiol or glycine was followed by observing the increase in absorbance at 291 nm (250 nm at pH 4, and 270 nm at pH 3.55 for thioglycolic acid reactions) due to the formation of the sustituted aniline chromophore. Reactions were followed using a modified Cary 17 UV-VIS spectrophotometer interfaced to an IBM 486 PC fitted with Olis software (Online Instrument Systems, Jefferson Ga., 1992). In all cases 3 ml of deoxygenated buffer was transferred via syringe to Ar flushed quartz cuvettes, followed by addition of 20-100 μL of a concentrated stock solution of the thiol (or glycine) in water or CH_3CN to give final concentrations of 2-15 mM (up to 3% CH_3CN). The cells were equilibrated in the spectrophotometer cell holder at 25.0°C for 10 min. after which the reaction was initiated by injection of 5-10 μL of a 0.08M stock solution of the amide in dry CH_3CN. An excess of thiol (glycine) of at least 10 fold was used so that the reactions were pseudo first order in all cases. Reactions were followed to at least 5 half times and runs were performed in duplicate at each catalyst concentration. Pseudo first order rate constants were obtained for each run by nonlinear least squares fitting of the absorbance vs. time data to a standard exponential model ($A_t = A_\infty + (A_o - A_\infty) \exp(-kt)$). The final pH of the cells were measured after each run to ensure constancy of pH, using a Radiometer Vit 90 Video

Titrator equipped with a GK2321C combination electrode standardized by Fisher certified pH 4.00, 7.00 and 10.00 buffers. Second order rate constants (k_2^{obs}) for the reaction of catalyst with amide **1** were obtained from the slopes of plots of k_{obs} vs. $[catalyst]_T$ at each pH (3 - 4 catalyst concentrations).

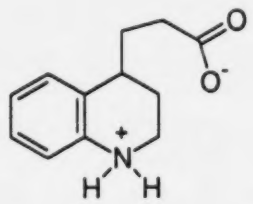
For the reactions of amide with thioglycolic acid at pH's 3.55, 4.05, 10.50, and 10.90, thioglycolate itself was used as the buffer. In these cases, the buffers were made up to the desired final concentrations (15 - 200 mM) by addition of thoroughly deoxygenated water to sodium thioglycolate and KCl in a volumetric flask, followed by rapid adjustment of pH using concentrated NaOH or HCl (O_2 free conditions). Three mL of the buffer solutions were then syringed into quartz cuvettes and runs were carried out as above. In cases where the absorbance of the buffer solution was greater than 2.0 due to the high concentration of thiol(ate) used, a cell containing buffer alone was placed into the reference compartment of the cell holder to subtract the background absorbance.

Thiol compounds were titrated periodically using either iodometric titration[5] or reaction with Ellman's reagent[6] (5,5′-dithiobis(2-nitrobenzoic acid)) in order to monitor the amount of air oxidation of thiol to disulfide.

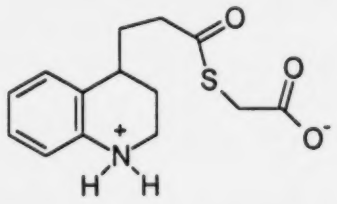
(D) Product Studies.

Amide 1 + Thioglycolic acid (2). In an attempt to isolate the product of reaction of amide **1** with thioglycolic acid, a large scale reaction was carried out in pH 7 phosphate buffer under conditions identical to those used in the kinetic runs (T = 25°C, μ = 1.0 (KCl)). The procedure followed is described below.

To a 200 mL volumetric flask was added 0.0767 g (0.00041 mol) of amide in 6 mL of CH_3CN followed by 0.53 g (0.0046 mol) of sodium thioglycolate. The solution was diluted to volume with pH 7.08 phosphate buffer (0.200 M, μ = 1.0 (KCl)) (solution was somewhat cloudy). The solution was stirred for 7.5 min.(~25 half times, where $t_{1/2} = \ln 2 / (k_2^{obs}[\text{thiol}]_T)$ and then 6 M HCl was added dropwise to bring the solution to pH 4.8. It was hoped that amino acid products such as **24** and **25** would exist in the zwitterionic form at this pH and could thus be extracted into organic solvent.



24

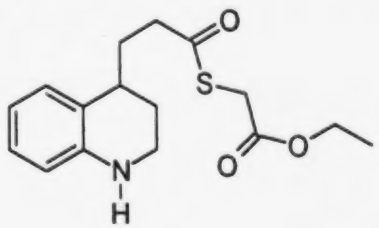


25

The aqueous solution was extracted with 3 x 60 ml of EtOAc and the combined extracts were dried over $CaCl_2$. After filtration and evaporation of the solvent 0.0493g of a yellow oil remained. Spectral data indicate that the major component isolated was thiol ester **25**. The characteristic carbonyl resonance for a thiol ester[42] was observed at 197.58 ppm in the ^{13}C spectrum as expected. Extra peaks present in the ^{13}C and ^{1}H NMR spectra not assignable to the thiol ester can be attributed to thioglycolic acid or its disulfide which were coextracted into the EtOAc. IR (CH_2Cl_2/MeOH cast film) ν 3100 - 2400 (NH_2^+), 1692 (S-C=O), 1603/1381(-COO^-), 1578, 1287, 1192 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ1.65-2.05 (m, 6H), 2.61-2.80 (m, 4H), 3.20 (s, HS-*CH_2*-COOH or disulfide), 3.13-3.32 (m), 3.30 (s, HS-*CH_2*-COOH or disulfide), 3.70 (s, 2H), 6.56 (d/d, J = 0.9,

8Hz, 1H), 6.62 (t/d, J = 0.9, 7.5 Hz, 1H), 6.92 (t/d, J = 1.3, 7.5 Hz, 1H), 6.98 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.55 (CH_2), 26.48 (CH_2, thioglycolic acid), 31.35 (CH_2), 31.42 (CH_2), 34.22 (CH), 38.85 (CH_2), 40.94 (CH_2), 117.39 (CH), 120.87 (CH), 127.00 (quat. C), 127.47 (CH), 129.32 (CH), 140.06 (quat. C), 174.02 (C=O,carboxylate), 176.32 (C=O, thioglycolic acid), 197.58 (C=O, thiol ester); HRMS (EI), exact mass calc'd for $C_{14}H_{17}O_3N_1S_1$ 279.0929, found 279.0921 (2.92%).

Amide 1 + Ethyl 2-mercaptoacetate (4). The product of reaction of amide **1** with ethyl 2-mercaptoacetate was isolated by the following procedure. To 26 mg ($1.39x10^{-4}$ mol) of amide in a 200 mL volumetric flask was added 6 mL of CH_3CN and 12 equivalents ($1.73x10^{-3}$ mol) of $HSCH_2COOEt$. The flask was quickly diluted to volume with deoxygenated water, and timing was started. The solution pH was adjusted to 7 with 4 M NaOH, and the reaction was allowed to proceed for 7 half times (based on k_2^{obs} = 0.34 $M^{-1}s^{-1}$ for the reaction of **1** and **4** at this pH). The reaction mixture was then worked up as above (except that $MgSO_4$ was used as a drying agent), giving 22 mg of a yellow oil. All spectral data were consistent with thiol ester **26** as the major product isolated.



26

The ^1H NMR spectrum corresponds to that of authentic **26** synthesized by an independent route (see below) + coextracted disulfide ($EtOC(O)CH_2SSCH_2C(O)OEt$). IR (CH_2Cl_2

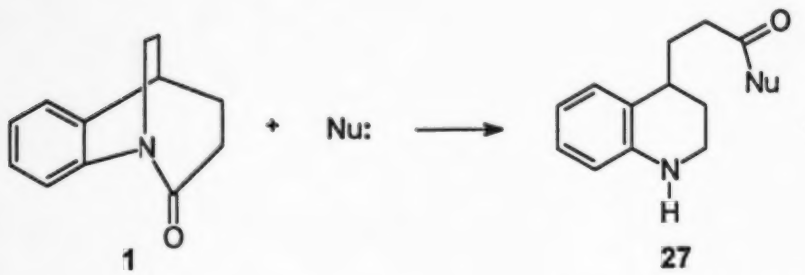
cast film) ν 3409 (NH), 2980, 2932, 2857, 1735 (C=O, O ester), 1695 (C=O, thiol ester), 1606, 1500, 1297, 1268 cm^{-1}; ^{13}C (75 MHz, $CDCl_3$) δ 14.13 (CH_3), 26.19 (CH_2), 31.34 (CH_2), 31.63 (CH_2), 34.77 (CH), 38.24 (CH_2), 41.22 (CH_2), 61.87 (CH_2), 114.27 (CH), 116.78 (CH), 123.66 (quat. C), 127.25 (CH), 129.17 (CH), 144.22 (quat. C), 168.77 (C=O, ester), 197.46 (C=O, thiol ester). Extra peaks in the ^{13}C spectrum can be identified as the disulfide of ethyl 2-mercaptoacetate; δ 14.18 ($-CH_3$), 41.53 ($-CH_2-SS-CH_2-$), 61.71 ($-CH_2-O-$), 169.37 (C=O). HRMS (EI), exact mass calcd. for $C_{16}H_{21}O_3N_1S_1$ 307.1242, found 307.1244 (31%).

Synthesis of Thiol Ester 26 by an Independent Route: To 3 mL of dry CH_3CN was added **1** (50 mg. 0.267 mmol), ethyl 2-mercaptoacetate (0.29 mL, 10 equiv.), and dry triethylamine (1 mL, 2.7 equiv.). After 24 hours of stirring at room temperature under Ar, volatiles were removed under vacuum (1mm Hg) and the resulting gum was triturated with hexane (3x25 mL) to remove the thiol ester. The hexane solutions were pooled and hexane removed to yield an oily yellowish product. This oil was dissolved in CH_2Cl_2 and washed several times with H_2O (3x50 ml) to further remove any contaminating thiolate or triethylammonium thiolate. The CH_2Cl_2 solution was dried over $MgSO_4$, and the CH_2Cl_2 removed in vacuo giving a yellowish oil. This oil was further purified by preparative TLC (Silica Gel 60 F_{254} plates, 50 EtOAc/50 Hexane) to give about 13 mg of product with the following characteristics: IR (CH_2Cl_2 cast film) ν 3410, 2979, 2928, 2856, 1737, 1694, 1606, 1500, 1474, 1298, 1264 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.28 (3H, t), 1.74-1.81 (1H, m), 1.87-1.99 (2H, m), 2.03-2.12 (1H, m), 2.66-2.85 (m, 3H), 3.25-3.26 (2H, m), 3.70 (2H, s), 3.90-4.20 (1H, br, s), 4.19 (2H, q), 6.48 (1H, d), 6.62 (1H, d), 6.96-7.01

(2H, m); ^{13}C NMR (75MHz, $CDCl_3$) δ 14.13, 26.15, 31.34, 31.61, 34.74, 38.22, 41.20, 61.88, 114.27, 116.79, 123.63, 127.25, 129.18, 144.19, 168.79, 197.51; HRMS, exact mass calcd. for $C_{16}H_{21}O_3N_1S_1$, 307.1242, found 307.1241 (42%).

Results

(A) Reaction of Thioglycolic Acid with Amide 1. The kinetics of the reaction of excess thioglycolic acid **(2)** with **1** were followed at 25.0°C (μ = 1.0 (KCl)) by observing at 291 nm (250 nm at pH 4.0, 270 nm at pH 3.5), the opening of the lactam ring to form the anilino derivative **27** (equation **(1)**).



1 **27** **(1)**

ε_{291} = 2200 $M^{-1}cm^{-1}$
(pH > 5)

The pseudo first order rate constants for ring opening (k_{obs}) were obtained at 4 different concentrations of thiol at each pH and apparent second order rate constants (k_2^{obs}) were then obtained from the slope of a plot of k_{obs} vs. $[thiol]_T$ according to the equation:

$k_{obs} = k_b + k_2^{obs}[thiol]_T$, where k_b = background rate constant for hydrolysis of **1** at a given pH. (See **Table** 1 for a collection of second order rate constants vs. pH.) The pH-rate constant profile (log k_2^{obs} vs. pH) for the reaction of thioglycolic acid with **1** is shown in **Fig.1.**

Table 1: Second Order Rate Constants Measured at Various pH Values for the Reaction of Thioglycolic Acid (**2**) with Amide **1** (T = 25.0°C, μ = 1.0 (KCl)).

pH	Buffer	k_2^{obs} ($M^{-1}s^{-1}$)[a]
3.55[b]	Thioglycolic acid	2.36 ± 0.36
4.00[b]	Thioglycolic acid	1.60 ± 0.15
4.50[c]	acetate	1.78 ± 0.18
5.16[d]	acetate	1.41 ± 0.02
5.40[c]	MES	1.52 ± 0.15
5.90[c]	MES	1.35 ± 0.04
7.00[c]	MOPS	1.73 ± 0.17
7.08[d]	phosphate	1.69 ± 0.18
7.94[c]	HEPES	1.65 ± 0.15
8.20[d]	HEPES	1.46 ± 0.08
9.00[c]	CHES	1.86 ± 0.04
10.50[b]	Thioglycolic acid	0.432 ± 0.003
10.90[b]	Thioglycolic acid	0.24 ± 0.03

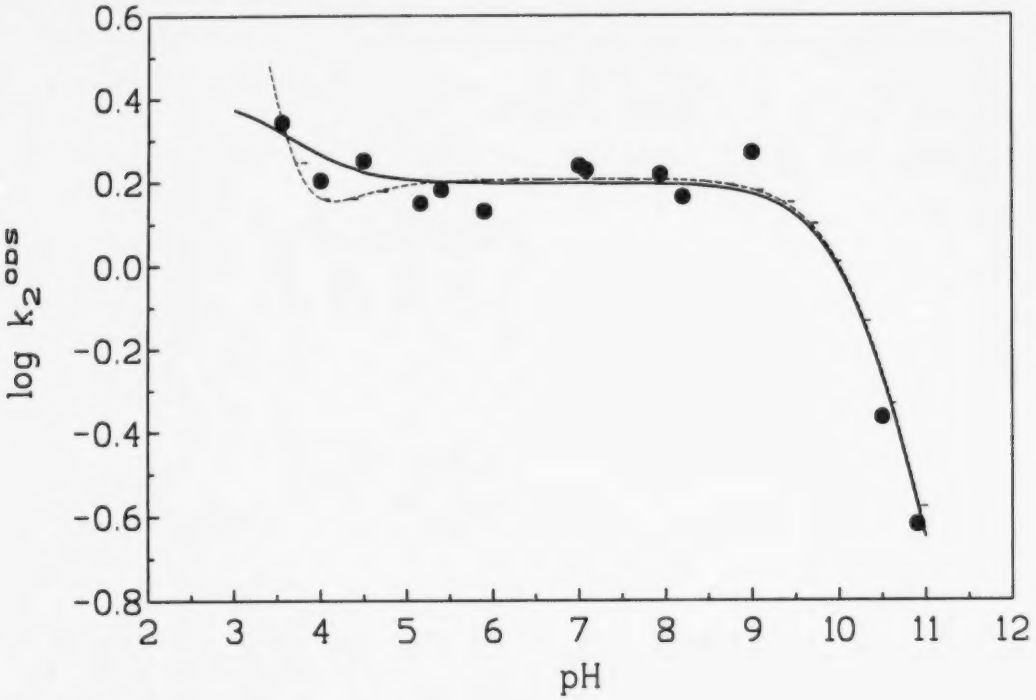
[a] k_2^{obs} determined from the slope of a plot of k_{obs} vs. $[thiol]_T$ at each pH (4 different thiol concentrations). The quoted error in k_2^{obs} is the std. dev. in the slope calculated by the linear regression fitting.

[b] Total buffer concentration was varied from 0.050-0.200 M.

[c] Total buffer concentration used = 0.050 M.

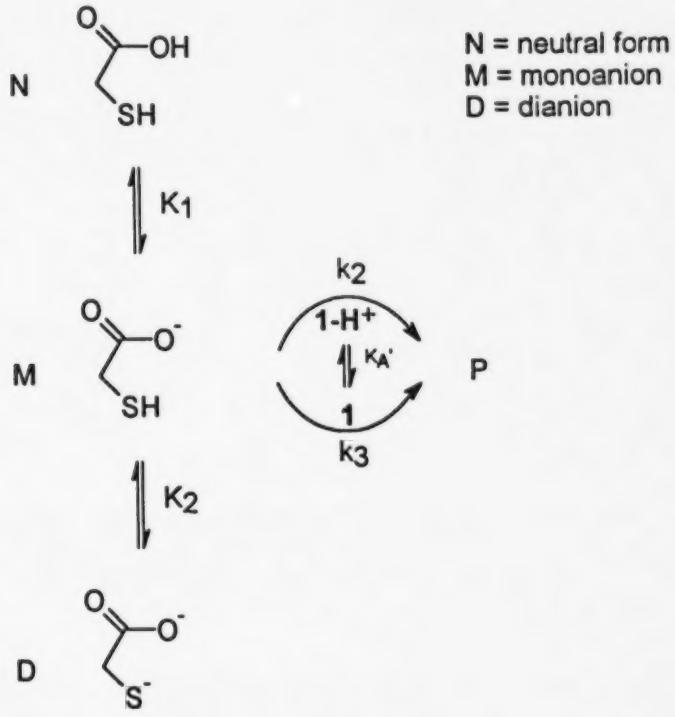
[d] Total buffer concentration used = 0.200 M.

Figure 1. Plot of log k_2^{obs} vs. pH for the reaction of **1** with **2**; T = 25.0°C, μ = 1.0 (KCl). Solid line (——) through the data computed on the basis of NLLSQ fit to equation **(5)**; dashed line (-----) through the data computed on the basis of NLLSQ fit to equation **(6)**.



This pH rate profile is consistent with the process shown in **Scheme 15** whereby the monoanionic form of thioglycolic acid (M) can react with both neutral and protonated amide.

Scheme 15:



From this scheme the following rate law for the appearance of ring opened product P, can be written (see Appendix 2 for a derivation):

(2) $d[P]/dt = -d[1]_T/dt = k_b[1]_T + k_2[M][1\text{-}H^+] + k_3[M][1]$

(3) $= \{k_b + (k_2\alpha_M\alpha_{1\text{-}H^+} + k_3\alpha_M)[RSH]_T\}[1]_T$

(4) $= (k_b + k_2^{obs}[RSH]_T)[1]_T$

where

$$(5) \qquad k_2^{obs} = \frac{(k_2/K_A')K_1[H^+]^2 + k_3K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2}$$

Here α_M = fraction of thiol present as the monoanion, $\alpha_{1\text{-}H^+}$ = fraction of amide in the protonated form, and $[RSH]_T$ = stoichiometric concentration of thioglycolic acid. K_1 and K_2 are the first and second acid dissociation constants for thioglycolic acid while K_A' is the acid dissociation constant for protonated amide.

Nonlinear least squares fitting of the data to this equation, after supplying values for K_1 $(2.82\text{x}10^{-4})$[39], K_2 $(6.02\text{x}10^{-11})$[39] and K_A' (2.08)[43], yielded the rate constants k_2 and k_3 given in **Table 2**. The solid curve through the experimental points shown in **Fig.1** was computed on the basis of the NLLSQ fit to eq. **(5)**. Fitting of the data to an equation including a term for attack of the dianion (D) on amide **1**, was also attempted, however the fit produced a negative value very close to zero for this rate constant, suggesting that this route to product is not important.

Table 2. Values for Rate Constants Derived from Nonlinear Least Squares Fitting of k_2^{obs} vs. $[H^+]$ Data for Thioglycolic Acid **(2)** to Equations **(5)** and **(6)**. [a,b]

Rate Constant	Equation	Value $(M^{-1}\ s^{-1})$[c]
k_2	5	$(1.9 \pm 0.2)\text{x}10^4$
k_3	5	1.58 ± 0.06
k_2'	6	$(2.1 \pm 0.3)\text{x}10^4$
k_3'	6	1.64 ± 0.06

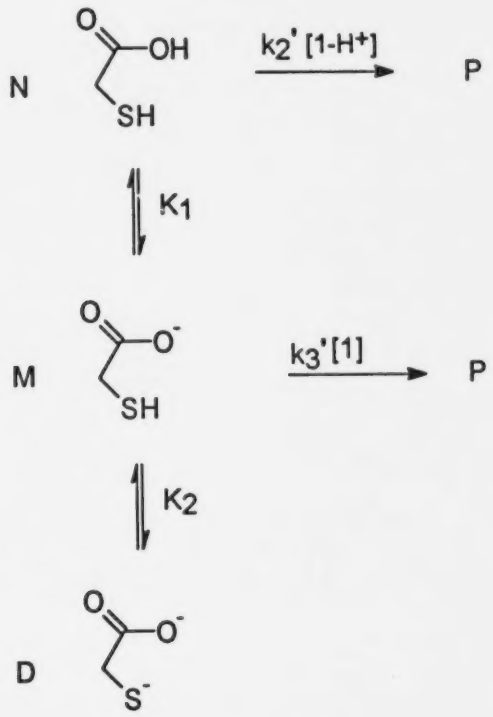
[a] k_2^{obs} vs. pH values given in Table 1.

[b] Literature values for K_1 $(2.82\text{x}10^{-4})$[39], K_2 $(6.02\text{x}10^{-11})$[39], and K_A' (2.08)[43], were input to the fitting program as constants.

[c] Quoted errors in rate constants are the standard errors obtained from the NLLSQ fit.

During the analysis of this pH-rate data it was realized that a second process, could also be used to fit the data. This process, shown in **Scheme 16** involves attack of the monoanion on **1** and attack of the neutral thiol-acid on **1-H$^+$**.

Scheme 16:



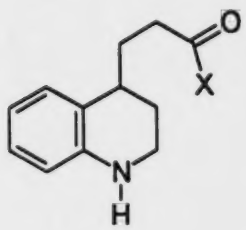
From this scheme can be derived equation **(6)** for k_2^{obs} (see Appendix 2 for a derivation).

(6)
$$k_2^{obs} = \frac{(k_2'[H^+]^3/K_A') + k_3' K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2}$$

Rate constants obtained from nonlinear least squares fitting of the data to equation **(6)** are given in **Table 2** and the theoretical curve calculated from the NLLSQ fit is shown as the dashed line through the points in **Fig.1**.

As shown in **Fig. 2**, a clear distinction between the 2 processes described by equations **(5)** and **(6)** can be made at pH's less than 3. Equation **(5)** (solid line, **Fig.2**), predicts a levelling off of log k_2^{obs} below pH 3, while equation **(6)** (dashed line **Fig.2**) predicts a linear increase in log k_2^{obs} below pH 3. Attempts were made to measure k_2^{obs} at pH 3, however because of the increasing rate of the background hydrolysis of **1** and the large absorbance of the thioglycolic acid buffers used at this pH, good rate constants could not be obtained. Therefore based on the experimental evidence alone we are unable to say whether the apparent increase in k_2^{obs} below pH 4 is due to attack of the monoanion (M) on protonated amide or to the attack of the neutral thiol on protonated amide.

One further point of interest should be noted with regard to the pH 4.05 data. These runs were followed by observing an increase in absorbance at 250 nm, even though the hydrolysis of amide to amino acid at this pH shows a decrease in absorbance at 250 nm. This is evidence that the formation of an intermediate other than the amino acid is being observed; probably thiol ester **25**, or anhydride **29**.

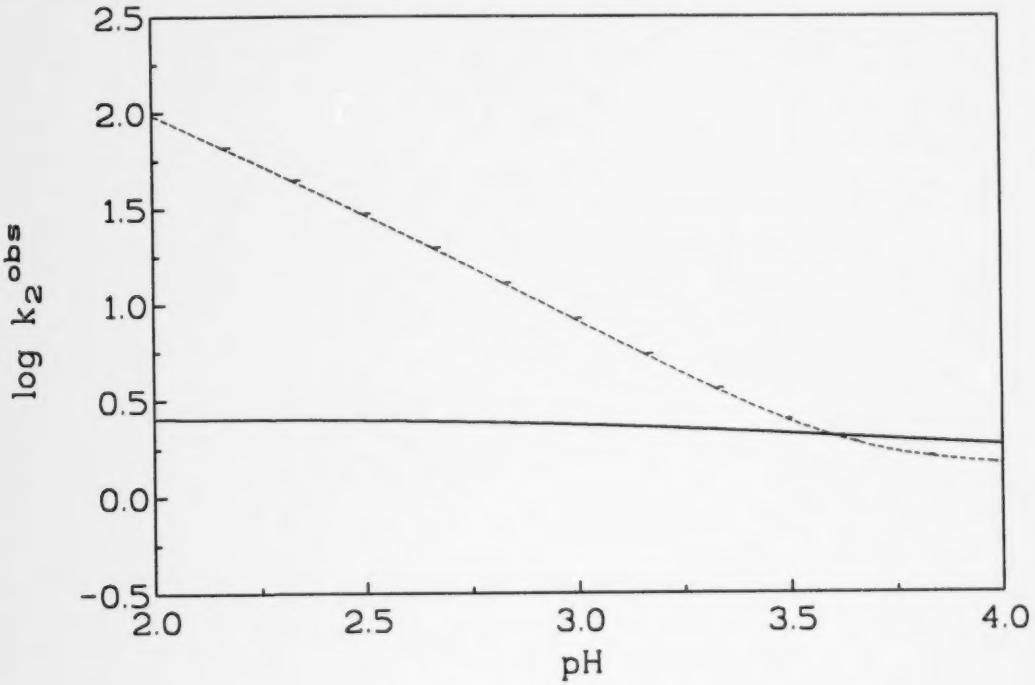


25: X = —SCH$_2$C(=O)OH (zwitterion form)

29: X = —OC(=O)CH$_2$SH

Evidence that thiol ester **25** is the product of the reaction at pH 7 was obtained by performing the reaction under standard kinetic conditions on a large scale and extracting the reaction mixture after about 25 half times to isolate potential products. Thiol ester **25** appeared to be the major compound isolated as judged by NMR and IR spectral data.

Figure 2. Plot of theoretical curve of log k_2^{obs} vs. pH for the reaction of **1** with thioglycolic acid (**2**) at low pH. Solid line (——) computed on the basis of NLLSQ fitting of the data to equation (**5**); dashed line (-----) computed on the basis of NLLSQ fitting of the data to equation (**6**).



(B) Reaction of Glycine with 1. Since the amino acid glycine **(3)** is isostructural with thioglycolic acid and has similar pK_A's (pK_1 = 2.34, pK_2 = 9.60)[44] it potentially should be able to catalyze hydrolysis of **1** in the same manner as thioglycolic acid. To test this hypothesis glycine was investigated as a catalyst for the ring opening of **1** at 25.0°C and μ = 1.0 (KCl). The second order rate constants measured at pH 6.18 and 7.55 are given in **Table 3.** Glycine was observed to catalyze the opening of **1**, however it is about 200 fold less effective as a catalyst than thioglycolic acid. The apparent second order rate constant (k_2^{obs}), appears to be independent of pH around neutrality as is the case for thioglycolic acid. It is possible then that glycine reacts with **1** via the same mechanism as thioglycolic acid; some possible explanations for the substantial rate difference observed will be discussed later.

Table 3: Second Order Rate Constants Measured at Various pH Values for the Reaction of Glycine **(3)** + Amide **1** (T = 25.0°C, μ = 1.0 (KCl)).

pH	buffer[a]	k_2^{obs} ($M^{-1}s^{-1}$)[b]
6.18	MES	$(6.5 \pm 0.1)\times10^{-3}$
7.55	MOPS	$(7.1 \pm 0.3)\times10^{-3}$

[a] $[Buffer]_T$ = 0.200M

[b] k_2^{obs} determined from the slope of a plot of k_{obs} vs. $[glycine]_T$ at each pH (4 different concentrations). The quoted error in k_2^{obs} is the std. dev. in the slope calculated by the linear regression fitting.

(C) Reaction of *cis* and *trans* 2-Mercaptocyclopentanecarboxylic Acids with 1. The rates of reaction of *cis* and *trans* 2-mercaptocyclopentanecarboxylic acids (**6** and **5**) with **1** were measured at 25.0°C, μ = 1.0 (KCl), over the pH range 5.0 - 7.5. The second order rate constants obtained are given in **Table 4.** Since the cis compound could not be completely separated from the trans compound, a 71/29 cis/trans mixture was used in the kinetic runs. The values for $k_2^{obs,cis}$ given in **Table 4** were indirectly obtained from a knowledge of $k_2^{obs,cis/trans}$ and $k_2^{obs,trans}$ at a given pH, according to eq. **(7)** (see Appendix 2 for a derivation).

(7) $$k_2^{obs,cis/trans} = 0.29k_2^{obs,trans} + 0.71k_2^{obs,cis}$$

As shown in **Table 4**, both the cis and trans compounds catalyze the opening of **1**, however there appears to be little significant difference in their catalytic abilities. For both the cis and the trans compounds, k_2^{obs} is found to increase as pH decreases from 7.49 to 5.08. Here again, a simplified mechanism involving attack of monoanion on neutral amide is not adequate to fit the data. The observed pH - rate data can be described by the process given in **Scheme 17**, which is exactly analogous to **Scheme 15** for thioglycolic acid, and involves attack of the monoanionic form of the catalyst on both the neutral and protonated amide.

Table 4: Second Order Rate Constants Measured at Various pH Values for the Reaction of *trans* and *cis* 2-Mercaptocyclopentanecarboxylic Acids (**5** and **6**) with Amide **1** (T = 25.0°C, μ = 1.0 (KCl)).

pH[a]	Buffer[b]	$k_2^{obs,\ trans}$ ($M^{-1}s^{-1}$)[c]	$k_2^{obs,cis/tr}$ ($M^{-1}s^{-1}$)[d]	$k_2^{obs,cis}$ ($M^{-1}s^{-1}$)[e]	($k_2^{obs,cis}$) / ($k_2^{obs,trans}$)
5.08	acetate	$(29 \pm 3)\times10^{-3}$	$(60 \pm 5)\times10^{-3}$	$(73 \pm 7)\times10^{-3}$	2.5±0.3
6.10	MES	$(11.0 \pm .7)\times10^{-3}$	$(17.1 \pm .5)\times10^{-3}$	$(21 \pm 2)\times10^{-3}$	1.9±0.2
6.89	MOPS	$(5.6 \pm .2)\times10^{-3}$	$(8.0 \pm .6)\times10^{-3}$	$(9.0 \pm .8)\times10^{-3}$	1.60±0.15
7.45	MOPS	$(4.9 \pm .4)\times10^{-3}$	$(5.7 \pm .1)\times10^{-3}$	$(6.0 \pm .6)\times10^{-3}$	1.20±0.15

[a] Error in pH of ± 0.04 units.

[b] $[Buffer]_T$ = 0.200 M

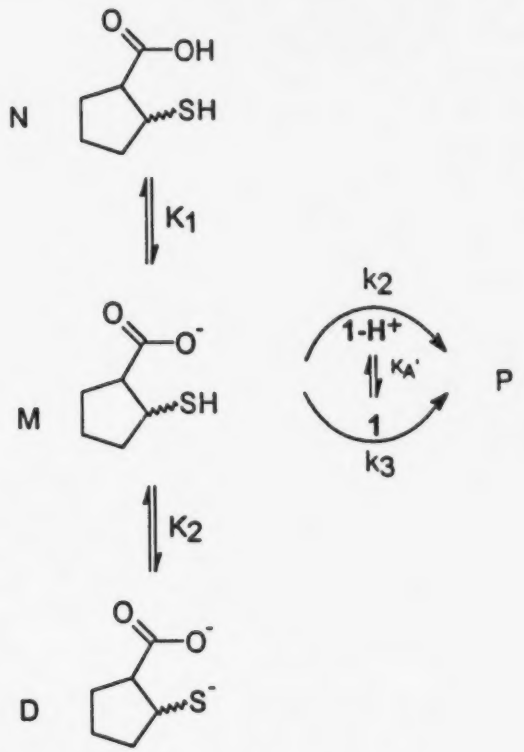
[c] $k_2^{obs,trans}$ determined from the slope of a plot of k_{obs} vs. $[thiol]_T$ at each pH (4 different thiol concentrations) for the trans thiol acid **5**. The quoted error in k_2^{obs} is the std. dev. in the slope calculated by the linear regression fitting.

[d] $k_2^{obs,\ cis/tr}$ determined from the slope of a plot of k_{obs} vs. $[thiol]_T$ where $[thiol]_T$ = the total concentration of a 71/29 mixture of cis/trans thiol.

[e] $k_2^{obs,cis}$ determined at a particular pH from the equation:

$k_2^{obs,cis/trans} = 0.29k_2^{obs,trans} + 0.71k_2^{obs,cis}$

Scheme 17:



The expression for k_2^{obs} derived from this scheme is given by equation **(5)**. However in the pH region between 5 and 7.5, eqn. **(5)** can be reduced to eqn. **(8)** by making the approximation that $([H^+]^2 + K_1[H^+]) >> K_1K_2$ (here we have estimated values for K_1 and K_2 to be those of 3-mercaptopropionic acid[45], $K_1 \approx 4.17 \times 10^{-5}$ and $K_2 \approx 4.17 \times 10^{-11}$).

(8)
$$k_2^{obs} = \frac{k_2K_1[H^+]/K_A' + k_3K_1}{K_1 + [H^+]}$$

Approximate values for the rate constants k_2 and k_3 can now be obtained by analysis of the data above pH 6 in the following way. If the assumption is made that the pK_A's of thiol

acids **5** and **6** are close to those of 3-mercaptopropionic acid, then at pH 6.10 and higher we can say that $(K_1 + [H^+]) \approx K_1$. Equation **(8)** then reduces to :

(9) $$k_2^{obs} = k_3 + k_2[H^+]/K_A'$$

Eq. **(9)** predicts that a plot of k_2^{obs} vs. $[H^+]$ should be linear with slope = k_2/K_A' and intercept = k_3. As shown in **Fig. 3**, plots of k_2^{obs} vs. $[H^+]$ (pH > 6) did prove to be linear. The values obtained for k_2 and k_3 from these plots are given in **Table 5**. From a comparison of k_3 for both the cis and the trans compound it appears that the magnitude of this rate constant for reaction of the monoanionic form of the thiol acid on the neutral amide is approximately the same for both compounds. This value of ~ 0.005 $M^{-1}s^{-1}$ may be compared to the corresponding rate constant for thioglycolic acid which has a value of 1.5 $M^{-1}s^{-1}$, some 300-fold larger. The values obtained for k_2, the rate constant for attack of monanion on protonated amide are of the same order of magnitude as the corresponding rate constant for thioglycolic acid (~ 10^4 $M^{-1}s^{-1}$).

Fig. 3. Plots of k_2^{obs} vs. $[H^+]$, pH > 6, for the reaction of cyclopentane thiol acids **5** and **6** with amide **1** (T = 25.0°C, μ =1.0 (KCl)); (O) trans thiol acid **5**, (□) cis thiol acid **6**.

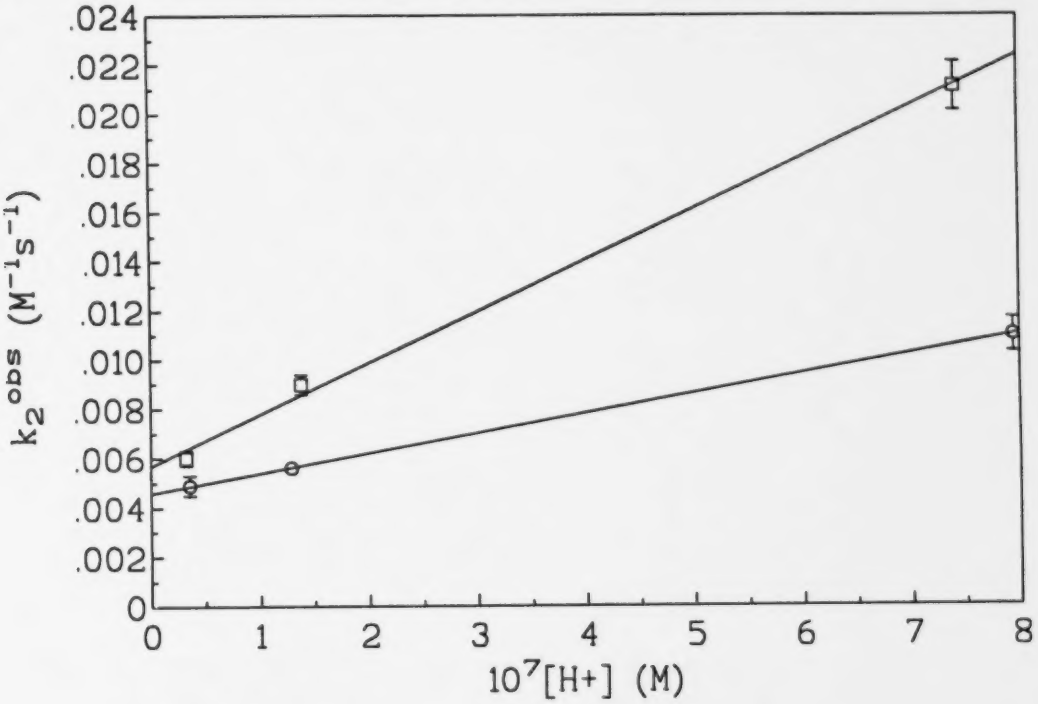


Table 5. Second Order Rate Constants for the Reaction of *trans* and *cis* 2-Mercaptocyclopentanecarboxylic Acids (**5** and **6**) with Amide **1** Obtained From Linear Regresssion Fitting of k_2^{obs} vs. $[H^+]$ to Equation (**9**) (pH > 6).

Compound	k_2 (M^{-1} s^{-1}) [a]	k_3 (M^{-1} s^{-1})
5 (trans)	$(1.7 \pm 0.1)x10^4$	$(4.59 \pm 0.03)x10^{-3}$
6 (cis)	$(4.3 \pm 0.3)x10^4$	$(5.7 \pm 0.4)x10^{-3}$

[a] k_2 obtained from slope = k_2/K_A' where K_A' = ionization constant of **1**-H^+ = 2.08 (ref. 43).

(D) Reaction of Ethyl 2-Mercaptoacetate with 1. The reactivity of ethyl 2-mercaptoacetate (**4**) towards amide **1** was investigated as a control experiment to verify that the high reactivity exhibited by thioglycolic acid towards **1** is dependent on both the thiol and carboxylic acid moieties.

During the collection of kinetic data on this system it was noted that if concentrations of amide exceeding ~ $1.3x10^{-4}$ M were used in the kinetic runs, the precipitation of some kind of intermediate occurred as the run proceeded. This intermediate is presumed to be the thiol ester **26** which could be isolated from preparative scale experiments (*vide infra*).

The pH rate profile for the reaction of **4** with **1** is given in **Fig. 4** (see **Table 6** for measured values of k_2^{obs} at each pH). As shown, the pH - rate profile appears to consist of a plateau region around neutrality with a downward bend occurring at pH ≈ pK_A^{SH} = 8.03[46]. The only mechanism which can account for this behaviour is one involving attack of thiolate ion on protonated amide (**Scheme 18**).

Table 6: Second Order Rate Constants Measured at Various pH Values for the Reaction of Ethyl 2-Mercaptoacetate (**4**) + Amide **1**[a] (T = 25.0°C, μ = 1.0 (KCl)).

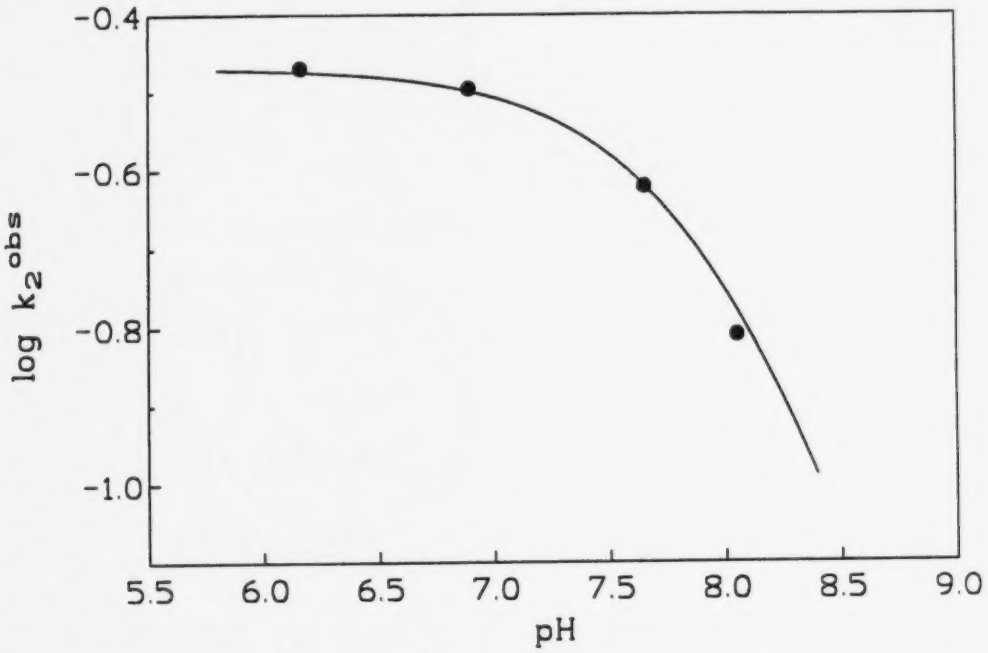
pH	Buffer[b]	k_2^{obs} ($M^{-1} s^{-1}$)[c]
6.16	MES	0.34 ± 0.03
6.89	MOPS	0.32 ± 0.01
7.65	MOPS	0.24 ± 0.02
8.05	HEPES	0.155 ± 0.007

[a] $[amide]_T = 1.3 \times 10^{-4}$ M in all runs.

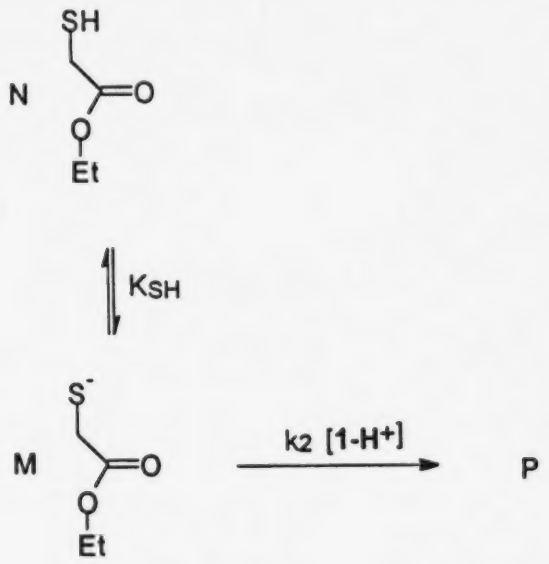
[b] $[Buffer]_T$ = 0.200 M

[c] k_2^{obs} determined from the slope of a plot of k_{obs} vs. $[thiol]_T$ at each pH (4 different concentrations). The quoted error in k_2^{obs} is the std. dev. in the slope calculated by the linear regression fitting.

Fig.4. Plot of log k_2^{obs} vs. pH for the reaction of ethyl 2-mercaptoacetate **(4)** with amide **1**, T = 25.0°C, μ = 1.0 (KCl). Solid line through the data computed on the basis of NLLSQ fitting of data to eqn. **(11)**.



Scheme 18:



The rate law for this process is given by:

(10) $$d[P]/dt = -d[1]_T/dt = (k_b + k_2^{obs}[RSH]_T)[1]_T$$

where

(11) $$k_2^{obs} = \frac{k_2 K_{SH}[H^+]}{K_A'(K_{SH} + [H+])}$$

(see Appendix 2 for a derivation). When nonlinear least squares fitting of the data to equation **(11)** was carried out after supplying values of $K_{SH} = 9.33 \times 10^{-9}$ (ref. 45) and $K_A' = 2.08$ (ref. 43) a value of $(7.6 \pm 0.5) \times 10^7$ $M^{-1}s^{-1}$ was obtained for k_2. This value seems consistent with previously measured rate constants for attack of other anionic species on protonated amide (**1-H$^+$**) when the nucleophilicity of the attacking group is taken into account.

The product of the reaction of thiol **4** with **1** at pH 7 was determined by carrying out the reaction on large scale (as described in the experimental section) followed by extraction of the pH 7 solution with EtOAc. The major product isolated was thiol ester **26**, as shown by a comparison of the NMR and IR spectral data with that of authentic **26** synthesized by an independent route. (Notably authentic **26** was found to be insoluble in aqueous buffers at a concentration of 2.5×10^{-4} M supporting the claim that this is the intermediate that precipitates out of solution in the kinetic experiments mentioned above where the [amide] was allowed to exceed 1.3×10^{-4} M.)

Discussion

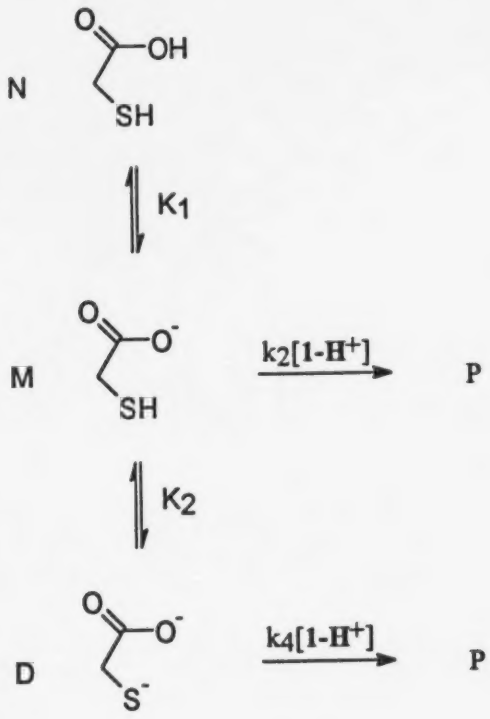
(A) Mechanism of Reaction of Thioglycolic Acid with Amide 1.

(i). Low pH region: As already discussed two processes can adequately fit the data for the reaction of thioglycolic acid with **1**. These are either; 1). (M + **1**) and (M + **1**-H^+); or 2). (M+1) and (N + **1**-H^+); where M and N indicate monoanionic and neutral forms of thioglycolic acid respectively. The most probable mechanism operating at pH < 4 that accounts for the observed rise in k_2^{obs} with decreasing pH is attack of the monoanion on protonated amide. The rate constant obtained for this process, $k_2 = 1.9 \times 10^4\ M^{-1}s^{-1}$ is of the same order of magnitude as that measured for the reaction of succinate monoanion with **1**-H^+ ($k_2 = 2.7 \times 10^4\ M^{-1}s^{-1}$)[29]. Furthermore, although there are some examples from the literature[47] where intramolecular addition of a neutral thiol to a protonated amide has been observed, there are apparently no examples of the corresponding intermolecular reaction. The rate constant determined from the NLLSQ fitting to eqn. **(6)** for the reaction of neutral thiol with protonated amide ($2.1 \times 10^4\ M^{-1}s^{-1}$) also seems much too high when the rate constant for the reaction of H_2O with 1-H^+ is taken into account ($k_2 = 2.4\ M^{-1}s^{-1}$)[43]. Based on these facts we feel the most likely process occurring at low pH is the attack of the monoanionic form of **2** on the protonated amide. Whether this attack involves the carboxylate or the thiolate as nucleophile (or a combination of both) is uncertain (*vide infra*).

(ii). pH > 4: Above pH 4 the predominant process appears to involve attack of the monoanionic form of thioglycolic acid on the neutral amide as shown by the pH independent region from pH 5-9 in the pH rate profile (**Fig.1**). A process which is

kinetically indistinguishable from attack of monoanion on neutral amide, attack of the dianion on the protonated amide, must also be considered here. It can be shown that the expression for k_2^{obs} for ring opening of **1** would be given by equation **(13)** if the mechanism involved (M + **1**-H^+) and (D + **1**-H^+) as shown in **Scheme 19** (see Appendix 2 for a derivation).

Scheme 19:



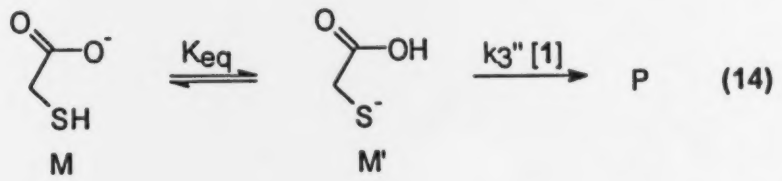
(13) $$k_2^{obs} = \frac{(k_2K_1[H^+]^2/K_A' + k_4K_1K_2[H^+]/K_A')}{[H^+]^2 + K_1[H^+] + K_1K_2}$$

Therefore k_3 obtained from NLLSQ fitting of the data to equation **(5)** would now be equated to k_4K_2/K_A', so that $k_4 = k_3K_A'/K_2 = 5.46 \times 10^{10}\ M^{-1}s^{-1}$. This rate constant may fall

at the higher end expected for a diffusion limited process[48] considering that the reaction involves the diffusion together of an anion of charge -2 and a cation of charge +1. As a result this mechanism cannot be completely ruled out. However the more favoured mechanism in the plateau region would be the attack of M on **1**. There are 3 different molecular mechanisms which must be considered for such a process.

1). Initial nucleophilic attack by the carboxylate followed by intramolecular general acid catalyzed breakdown of the tetrahedral intermediate by the pendant thiol **(Scheme 20)**. The linear anhydride **30** (which is analogous to one previously shown to be too unstable to detect in solution)[29] is rapidly captured by internal cyclization to form the thiol ester. Such a process is very similar to the one proposed for the reaction of dicarboxylic acid monoanions with **1**[29].

2). A second potential mechanism could involve attack of the small equilbrium concentration of thiolate monoanion on amide **1**, equation **(14)**.

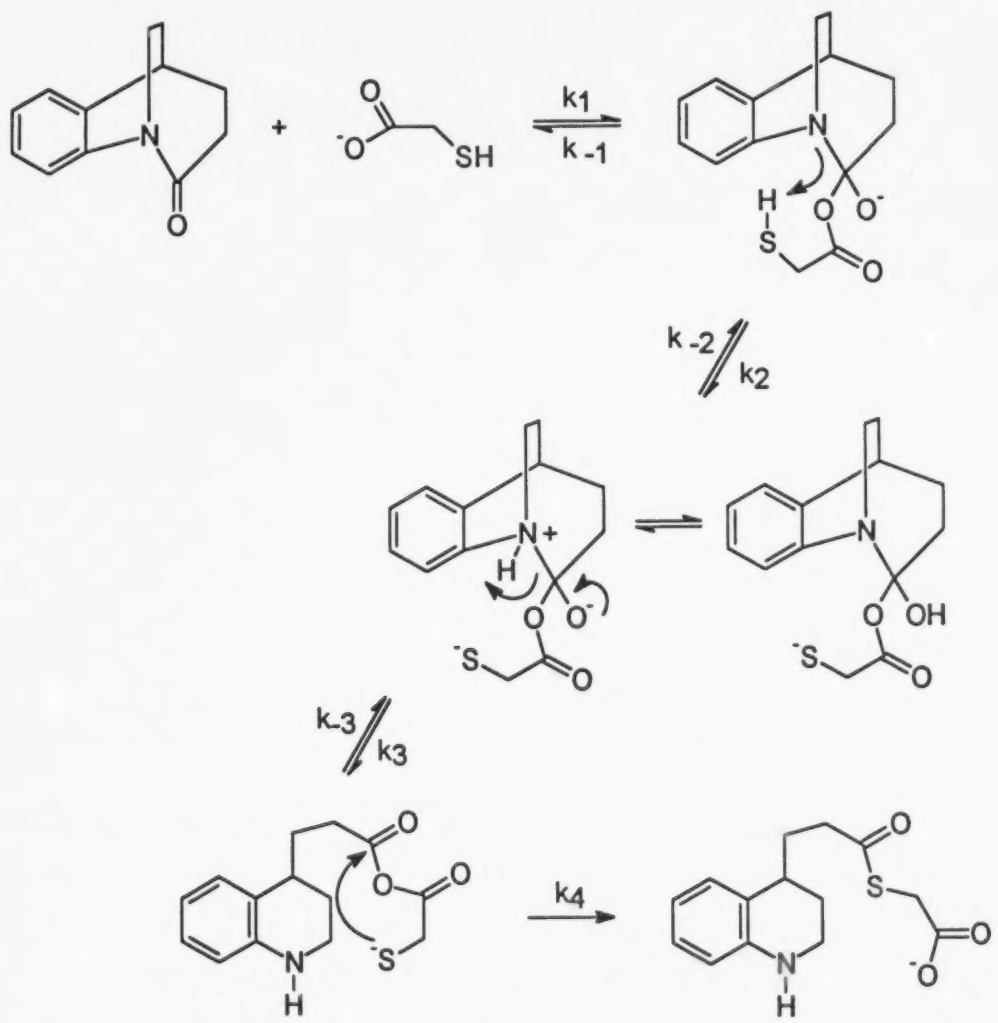


(14)

The value for k_3 obtained from NLLSQ fitting of the data to equation **(5)** must now be equated to $k_3''K_{eq}$. A value for K_{eq} of 3.55×10^{-5} can be estimated from an examination of the thermodynamic cycle shown in Appendix 2. This gives a value of $k_3'' = k_3/K_{eq} = 4.45 \times 10^4\ M^{-1}s^{-1}$ for the rate of attack of the thiolate monoanion on **1**. The molecular mechanism for this process would involve attack of the thiolate on amide to form the

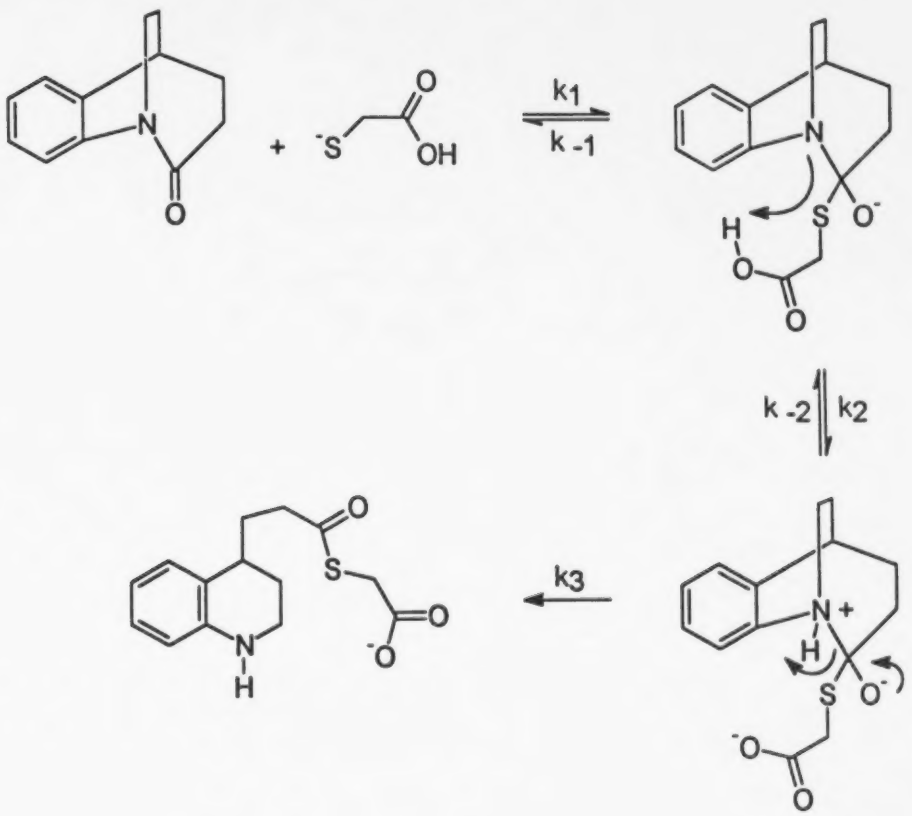
tetrahedral intermediate, followed by proton trapping of this intermediate by the pendant carboxyl group **(Scheme 21)**.

Scheme 20:

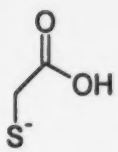


30

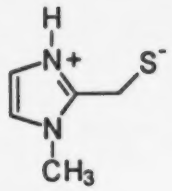
Scheme 21:



If the mechanism does involve attack by the thiolate with subsequent general acid catalyzed breakdown of the tetrahedral intermediate by the pendant -COOH, then one must explain why this process is 400 times faster than the reaction of 2-(mercaptomethyl)-N-methylimidazole **(18)** with **1** (k_2 = 100 $M^{-1}s^{-1}$). One possibility is that the increased reactivity of **2** is due to the greater nucleophilicity of thiolate **2a** ($pK_A \approx 8.0$, estimated from the pK_A of $HSCH_2COOEt$[46]) than thiolate **18a** ($pK_A = 6.4$)[31].



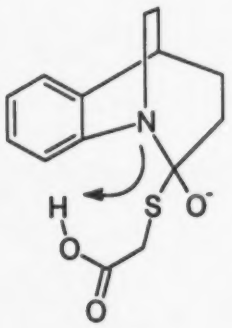
2a
$pK_a(SH) = 8.0$



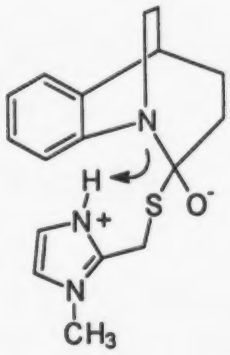
18a
$pK_a(SH) = 6.4$

Jencks showed[49] that for reactions involving thiolate attack on O esters where breakdown of the tetrahedral intermediate is the rate limiting step, that the Brønsted β lies in the range of 0.8 to 1.0 ($\log k_2 = \beta pKa + C$; where k_2 is the 2nd order rate constant for the reaction of thiolate with ester). If the Brønsted β for attack of thiols on amide **1** is ~ 1, then an increase in pK_A of ~ 2 units in going from thiolate **18a** to thiolate **2a** could lead to a 50-100 fold increase in the observed second order rate constant.

A second possible source of the enhanced reactivity of **2a** could be the more facile proton transfer that would be possible in tetrahedral intermediate **A** relative to **B**.



A



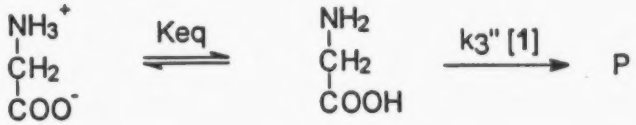
B

The proton transfer process depicted in **A** will be more thermodynamically favourable (pK_A (COOH) ≈ 3.5, pK_A (anilino N) ≈ 7-8) than that depicted in **B** (pK_A (imidazolium) ≈

7-8). If proton transfer is partly rate limiting then this could contribute to the observed rate difference for **2a** relative to **18a**.

The observation that glycine is a very poor catalyst for ring opening of **1** compared to thioglycolic acid, even though it is nearly isostructural with **2** and has very similar pK_A's may be consistent with the mechanism proposed here (ie. **Scheme 21**). If it is assumed that a similar mechanism is operating in the reaction of glycine with **1**, as shown in Equation **(15)**, then a value of $k_3'' = 1.9x10^3\ M^{-1}s^{-1}$ can be obtained by an analysis identical to that used for thioglycolic acid (see Appendix 2 for details).

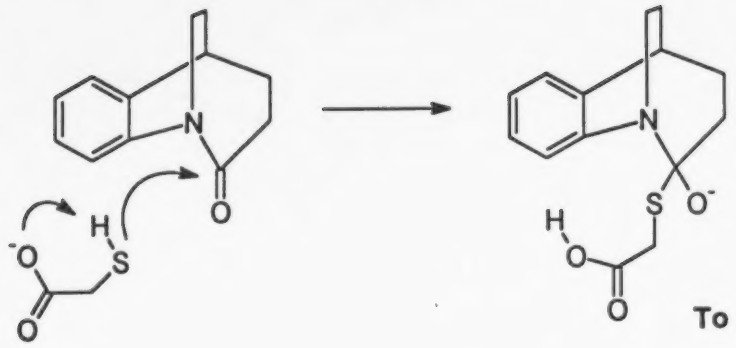
(15)



The low value of k_2^{obs} for glycine (pH 7) could then be attributed to a combination of two factors. The first is the less favourable equilibrium constant ($K_{eq} \approx 3.5x10^{-6}$) that results in a smaller concentration of the active amino-carboxylic acid form in solution. The second is the smaller value for the rate constant k_3'' which can probably be attributed to the greater nucleophilicity of a thiolate relative to an amine of identical pK_A towards acyl carbon. For example Jencks has shown that $HOCH_2CH_2S^-$ (pK_A SH = 9.5) is an order of magnitude more reactive towards pNPA than is glycine, $NH_2CH_2COO^-$, ($pK_A\ NH_3^+ = 9.6$)[50].

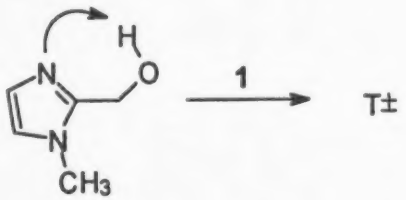
3). A third mechanism which could be postulated for the reaction of the monoanionic form of thioglycolic acid with **1** involves the intramolecular general base catalyzed attack of thiol on amide as shown below.

(16)



(To^- could then break down with intramolecular general acid assistance as in **Scheme 20**). The only real evidence against such a mechanism is the lack of precedent in the literature for general base catalyzed attack of thiols on carbonyl compounds[51]. However it should be noted that an unfavourable intermolecular process can become a favourable intramolecular process[52]. A similar mechanism involving intramolecular general base catalysis has been proposed for the reaction of various β-amino alcohols with **1**[27b].

(17)



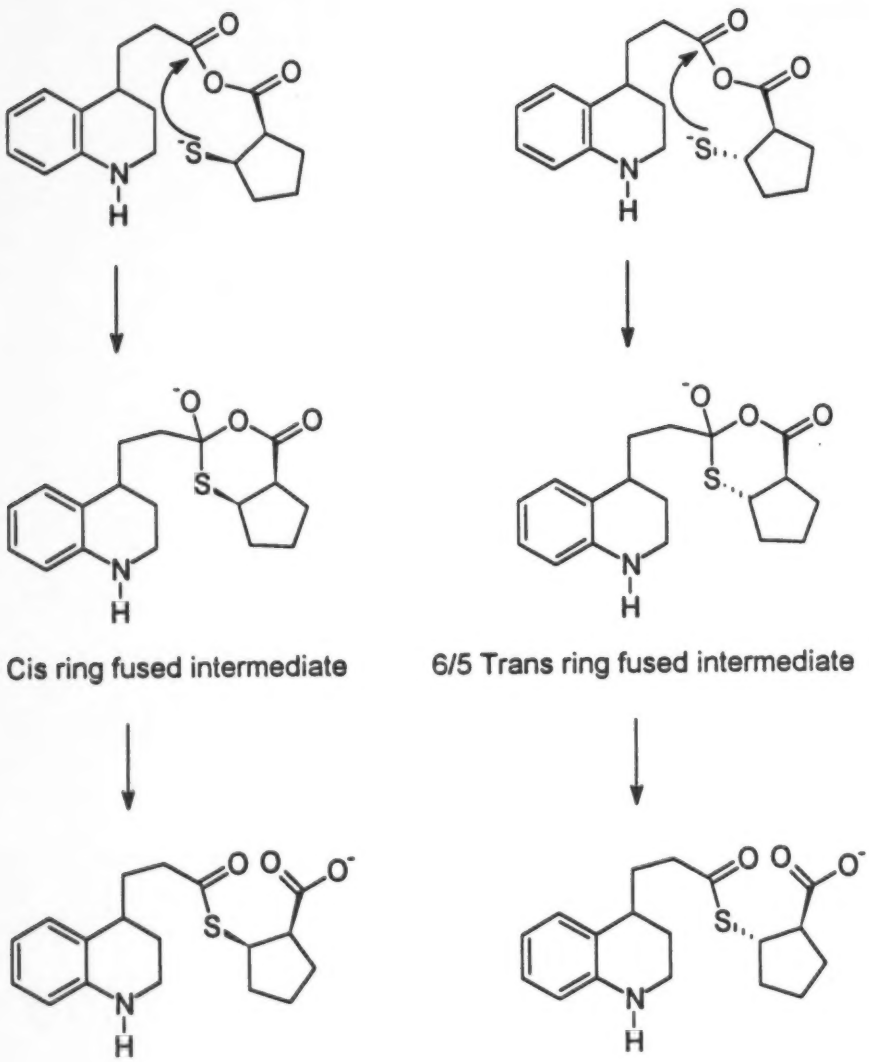
The difference in pK_A of the H^+ donor and acceptor groups in this example is very similar to that found in thioglycolic acid ($\Delta pK_A \sim 7$). As a result a mechanism involving intramolecular general base catalysis probably cannot be completely ruled out for the reaction of **M + 1**.

(B) Mechanism of Reaction of *cis* and *trans* 2-Mercaptocyclopentane-carboxylic Acids with 1. The observed pH rate data for trans and cis thiol acids **5** and **6** with **1** can also be fit adequately by a process involving attack of monoanion on **1** coupled with attack of monoanion on **1-H$^+$**. Thus it appears that there may be a consistent mechanism for the reaction of these thiol acids with **1**.

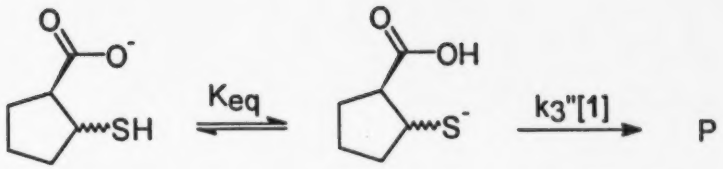
As shown in **Table 5** the rate constants for the attack of the monoanionic form of the cis or trans cyclopentane thiol acids on amide **1** are approximately the same (k_3 = 0.0057 and 0.0046 $M^{-1}s^{-1}$ respectively). Our original expectation was that if these thiol acids reacted with **1** via initial attack of the carboxylate to form an open anhydride followed by internal cyclization to the thiol ester **(Scheme 20)**, then the cis compound would be a more efficient catalyst than the trans. This is because internal cyclization of the anhydride formed from the cis compound would lead to formation of a cis 6-5 ring fused intermediate whereas internal cylization of the anhydride formed from the trans compound would lead to a more unfavourable trans 6-5 ring fused intermediate **(Scheme 22)**. (The thermodynamic instability of trans 6/5 ring fused derivatives is demonstrated by the well known reluctance of trans vicinal diols of glycopyranosides to form cyclic acetals[53].)

The fact that there is no significant difference in k_3 for the cis and trans compounds suggests that either the internal cyclization step is not rate limiting or that an alternative mechanism is operative. The most obvious alternative mechanism would be attack of the thiolate monoanion on **1** (equation **(18)**), in a manner exactly analagous to that shown in **Scheme 21**.

Scheme 22:



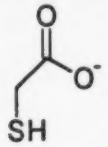
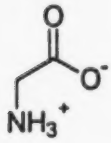
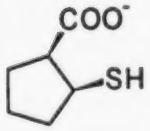
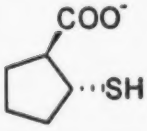
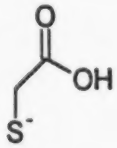
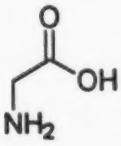
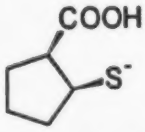
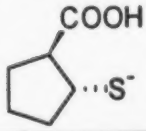
Such a mechanism would bypass the internal cylization step since the thiol ester is formed directly. This could then account for the small difference in rate of reaction of **5** and **6** monoanion with **1**.

(18) 

In this case k_3 obtained from linear regression fitting of the pH-rate data to equation **(9)** would now be equated to $k_3''K_{eq}$. K_{eq} can be estimated as 7.8×10^{-6} (see Appendix 2) using the same kind of analysis as used previously, giving a value for k_3'' of 5.9×10^2 $M^{-1}s^{-1}$ for the trans compound and 7.3×10^2 $M^{-1}s^{-1}$ for the cis compound . These numbers can be compared to a value 4.45×10^4 $M^{-1}s^{-1}$ for the corresponding rate constant for the attack of $HOOC\text{-}CH_2\text{-}S^-$ on **1**. The difference in these values may be due to a more sterically unfavourable attack step for the cyclopentane thiol acids coupled with a less favourable proton transfer from the pendant -COOH to the N of the tetrahedral intermediate. (It has already been suggested that the intramolecular proton transfer to the N of the tetrahedral intermediate may be part of the rate limiting step if reaction occurs by attack of the thiolate form of the monoanion (vide supra).)

(A summary of all of the calculated rate constants for reaction of the various bifunctional species with neutral **1** is given in **Table 7**.)

Table 7. Rate Constants Calculated for the Reaction of Various Bifunctional Species with Neutral **1**.

Compound	Approx. pK_A of nucleophilic group	k_2 ($M^{-1}s^{-1}$)
	3.55[a]	1.58
	2.34[b]	0.0070
	4.4[c]	0.0057
	4.4[c]	0.0046
	8.0[d]	4.45×10^4
	7.8[e]	1.9×10^3
	9.4[f]	730
	9.4[f]	590

[a] Ref. 39.

[b] Ref. 44.

[c] Estimated as pK_1 of 3-mercaptopropionic acid, ref. 45.

[d] Estimated as the pK_A os $HSCH_2COOEt$, ref. 46.

[e] Estimated as the pK_A of H_2NCH_2COOEt, Ref. 57.

[f] Estimated as the pK_A of $HSCH_2CH_2COOEt$, ref. 58.

(C) Mechanism of Reaction of Ethyl 2-Mercaptoacetate (4) with 1.

The ethyl ester of thioglycolic acid appears to react with **1** exclusively through a mechanism involving attack of the thiolate anion on the protonated amide. The fact that the thiolate anion of **4** is unreactive towards the unprotonated amide supports a mechanism of reaction of thiol acids **2**, **5** and **6** with neutral **1** that requires both the thiol and carboxylic acid moieties for activity.

(D) Reactions of Thiol Acids with the Activated Ester pNPA.

There are a few examples in the literature of the reaction of thiolacids with the activated ester pNPA. Bender and Schonbaum[34] studied the reaction of o-mercaptobenzoic acid with pNPA. From the results of a pH-rate study they concluded that the active form of the catalyst was the dianion and that the reaction involved attack of the thiolate center on pNPA. A study by Jencks and Carriuolo[30] has shown that thioglycolic acid reacts with pNPA via attack of the thiolate group of the dianion. Thiol acids thus appear to react with pNPA and with amide **1** by different routes. A similar difference in the mode of reactivity of 2-(mercaptomethyl)-N-methylimidazole **(18)** with amide **1** and pNPA has been noted in a previous study[35]. In that study it was found that the monoanion was the most active form of the catalyst towards pNPA while the zwitterionic ammonium

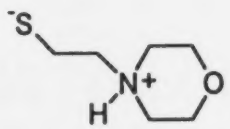
thiolate was the most active form towards amide **1**. These studies demonstrate that distorted amide **1** and probably amides in general have different catalytic requirements for cleavage than do activated esters such as pNPA. This difference arises from the need to protonate the leaving N of the tetrahedral intermediate, formed upon nucleophilic attack on an amide. An activated ester such as pNPA has no such requirements since the weakly basic leaving group can be easily expelled from the tetrahedral intermediate without assistance. Whether a nonactivated ester such as methyl acetate has catalytic requirements that are closer to that of an activated ester or to that of an amide is a question that has not been much addressed to date, as most studies on catalyzed ester hydrolysis have involved activated esters. However a few preliminary reports suggest that the catalyzed hydrolysis of nonactivated esters may, like amides, require some kind of facilitation of leaving group expulsion by the catalyst for maximal activity[56]. (In this regard it might be of interest to see whether the zwitterionic imidazolium thiolate **18a** is more reactive than the more basic monoanion of **18**, towards a nonactivated ester like methyl benzoate.)

Conclusions

In this study a series of thiol acids have been shown to be effective agents for the ring opening of distorted amide **1**. On comparing all of the bifunctional catalysts that have been studied to date, thioglycolic acid is only bettered as a catalyst for ring opening of amide **1** at neutral pH's by 2-mercaptomethyl-N-methylimidazole **(18)**, the advantage of this catalyst being the large concentration of the active zwitterionic ammonium thiolate that is present at neutral pH. The pH-rate data for the thiol acids indicate that the 2 predominant modes of reaction with **1** involve attack of the monoanionic form of the thiol acid on the neutral amide and attack of the monoanion on the protonated amide. The mechanism proposed here for the reaction of thiol acids **2**, **5** and **6** with neutral **1**, requiring both a nucleophilic group and an acidic functionality for the trapping of the tetrahedral intermediate, is entirely consistent with mechanisms postulated previously for the reaction of dicarboxylic acids, β-amino alcohols and ammonium thiolates with **1**. Whether the mechanism involves initial attack by carboxylate anion as shown in **Scheme 20** or initial attack by the thiolate monoanion as shown in **Scheme 21,** is not readily determinable. However the data collected here can be more easily explained as a whole if the mechanism of reaction of monoanionic thiol acids with **1** involves attack of the anionic thiolate form.

Future Studies

Identification of the Rate Limiting Step. The question of which step is rate limiting in the reaction of thiol acids **2**, **5** and **6** with **1** can not be determined from the data presented here. To determine if the intramolecular proton transfer to the N of the tetrahedral intermediate (k_2, **Schemes 20** or **21**) is part of the rate limiting step a study of the reaction of thioglycolic acid with **1** in the presence of varying concentrations of N-methyl imidazole buffer can be carried out. If proton transfer were part of the rate limiting step it might be possible to detect buffer catalysis of k_2^{obs} (i.e. intermolecular general acid catalysis of the breakdown of the tetrahedral intermediate by N-methyl imidazolium ion) and possibly saturation kinetics in the plot of k_2^{obs} vs. $[buffer]_T$. It has already been shown, in the case of the reaction of the zwitterion of 4-(2-mercaptoethyl)morpholine **(31)** with **1**, that intermolecular general acid catalysis by N-methylimidazole is detectable, suggesting that intramolecular proton transfer to the N of the tetrahedral intermediate is partially rate limiting in this case[31].

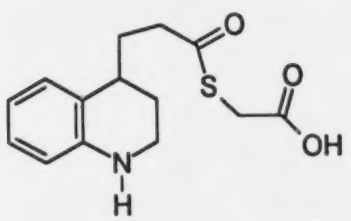


31

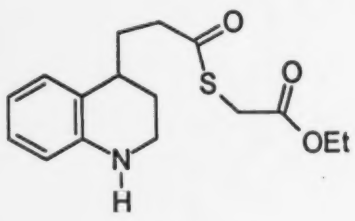
However, when similar experiments were carried out with 2-(mercaptomethyl)-N-methylimidazole **(18)** no buffer catalysis of k_2^{obs} was detectable. It should be noted however that the absence of observable buffer catalysis cannot be used to rule out rate limiting proton transfer since it may simply imply that intermolecular trapping of the

tetrahedral intermediate by buffer acid cannot compete with the more efficient intramolecular process.

Determination of the Hydrolytic Profile of Thiol Ester Products. Since the product of the reaction of thioglycolic acid with **1** was shown to be the thiol ester **25**, it is evident that the deacylation of this thiol acid is slow relative to its acylation.



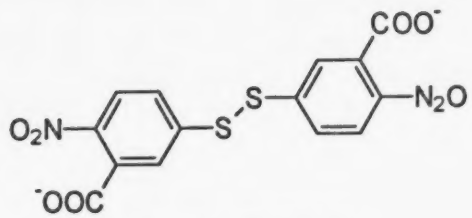
25



26

In order to determine the time scale required for turnover of this 'catalyst', the rate of hydrolysis of the thiol ester must be determined. In this regard it would be interesting to obtain the hydrolytic profiles of both thiol ester **25** and **26**. A comparison of these 2 profiles would indicate if the pendant carboxylate provides any assistance in the hydrolysis of thiol ester **25**.

Preliminary experiments indicate that it may be difficult to follow the hydrolysis of these thiol esters by UV-Vis spectrophotometry due to lack of an observable absorbance change. If this is the case then it may be possible to follow the reaction by monitoring the realease of thiolate ion by including Ellmans reagent (5,5'-dithiobis(2-nitrobenzoic acid) in the reaction medium, as was done in a previous study[32].



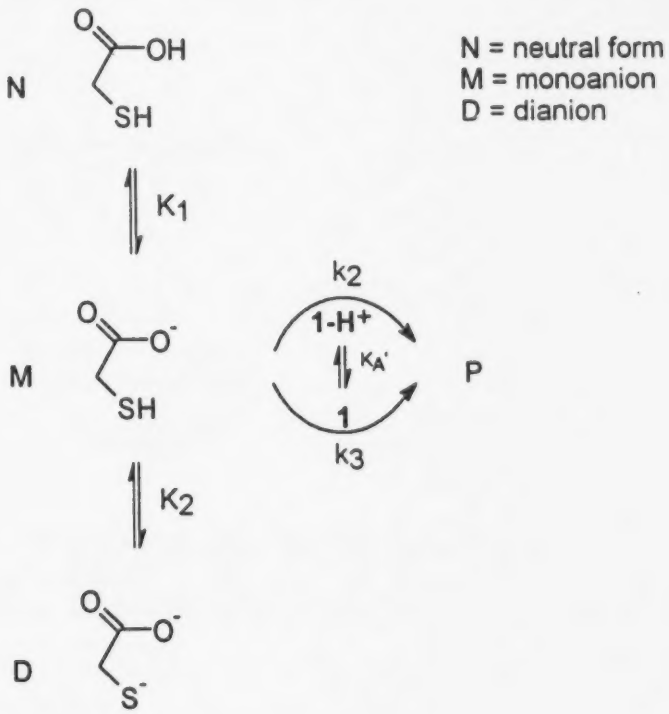
Ellmans Reagent

Thiolate produced in the hydrolysis reaction will react rapidly with this disulfide to produce the highly absorbing thiophenoxide species, 5-mercapto(2-nitrobenzoic acid). The appearance of this species can be monitored by following the reaction at 412 nm[41].

APPENDIX 2 - Supplementary Material to Part 2

Derivation of equation (5) for the process shown in Scheme 15.

Scheme 15:



$d[P]/dt = -d[\mathbf{1}]_T/dt = k_b[\mathbf{1}]_T + k_2[M][\mathbf{1}\text{-}H^+] + k_3[M][\mathbf{1}]$

at $pH > 3$, $[\mathbf{1}] \approx [\mathbf{1}]_T$

$d[P]/d[t] = k_b[\mathbf{1}]_T + k_2\alpha_M[RSH]_T\alpha_{1\text{-}H^+}[\mathbf{1}]_T + k_3\alpha_M[RSH]_T[\mathbf{1}]_T$

where $\alpha_M = [M]/[RSH]_T$ = the fraction of thioglycolic acid present as the monoanion and $\alpha_{1\text{-}H^+} = [\mathbf{1}\text{-}H^+]/[\mathbf{1}]_T$ = the fraction of amide present as the protonated form.

$d[P]/dt = (k_b + k_2^{obs}[RSH]_T)[\mathbf{1}]_T = k_{obs}[\mathbf{1}]_T$

where $k_2^{obs} = k_2\alpha_M\alpha_{1\text{-}H^+} + k_3\alpha_M$

$\alpha_M = [M]/([M] + [N] + [D])$

$K_1 = [M][H^+]/[N]$; $K_2 = [D][H^+]/[M]$

so that:

$[N] = [M][H^+]/K_1$; $[D] = K_2[H^+]/[M]$

$\alpha_M = K_1[H^+]/([H^+]^2 + K_1[H^+] + K_1K_2)$

{In general for a diprotic acid, H_2A, it can be shown that:

$\alpha_{H_2A} = [H^+]^2/([H^+]^2 + K_1[H^+] + K_1K_2)$; $\alpha_{HA^-} = K_1[H^+]/([H^+]^2 + K_1[H^+] + K_1K_2)$

and $\alpha_{A_=} = [H^+]^2/([H^+]^2 + K_2[H^+] + K_1K_2)$ }

$\alpha_{1\text{-}H^+} = [\mathbf{1}\text{-}\mathbf{H}^+]/[\mathbf{1}]_T$

$K_A' = [\mathbf{1}][H^+]/[\mathbf{1}\text{-}\mathbf{H}^+] = \{([\mathbf{1}]_T - [\mathbf{1}\text{-}\mathbf{H}^+])[H^+]\}/[\mathbf{1}\text{-}\mathbf{H}^+]$

Therefore $\alpha_{1\text{-}H^+} = [\mathbf{1}\text{-}\mathbf{H}^+]/[\mathbf{1}]_T = [H^+]/(K_A' + [H^+])$

{ In general for a monoprotic acid HA: $\alpha_{HA} = [H^+]/(K_A' + [H^+])$ and

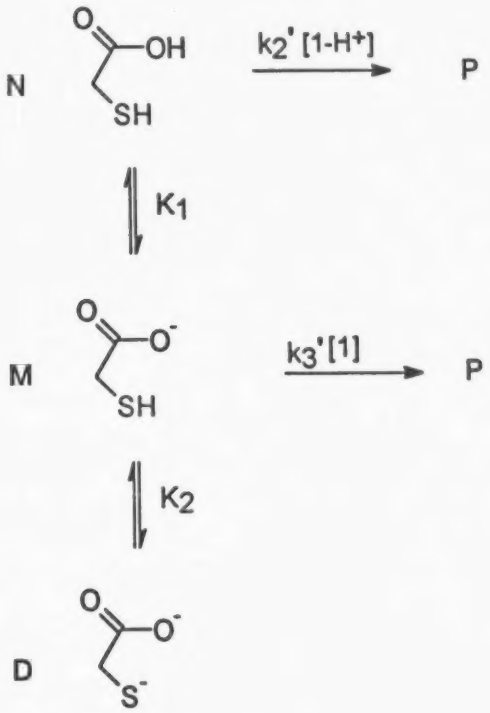
$\alpha_{A^-} = K_A'/(K_A' + [H^+])$ }

Substituting these expressions for α_M and $\alpha_{1\text{-}H^+}$ into the expression for k_2^{obs} and making the assumption that $K_A' + [H^+] \approx K_A'$ (pH > 3 since $K_A' = 2.08$)[43], gives:

$$\textbf{(5)} \qquad k_2^{obs} = \frac{\left(k_2/K_{A'}\right)K_1[H^+]^2 + k_3K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2}$$

Derivation of Equation (6) for the process shown in **Scheme 16**

Scheme 16:



$$d[P]/dt = -d[1]_T/dt = k_b[1]_T + k_2'[N][1\text{-}H^+] + k_3'[M][1]$$

$$= (k_b + (k_2'\alpha_N\alpha_{1\text{-}H^+} + k_3'\alpha_M)[RSH]_T)[1]_T = (k_b + k_2^{obs}[RSH]_T)[1]_T$$

where $k_2^{obs} = k_2'\alpha_N\alpha_{1\text{-}H^+} + k_3'\alpha_M$

The following expressions for α_N, α_M, and $\alpha_{1\text{-}H^+}$ can be derived from the expressions for K_1, K_2 and K_A' as already described for the derivation of eq. (5)

$$\alpha_N = [H^+]^2/([H^+]^2 + K_1[H^+] + K_1K_2)$$

$$\alpha_M = K_1[H^+]/([H^+]^2 + K_1[H^+] + K_1K_2)$$

$\alpha_{1\text{-}H^+} = [H^+]/(K_A' + [H^+])$

Substitution of these expressions into the expression for k_2^{obs} and making the approximation that $K_A' + [H^+] \approx K_A'$ (pH > 3) gives:

$$(6) \qquad k_2^{obs} = \frac{\left(k_2'[H^+]^3 / K_A'\right) + k_3' K_1 [H^+]}{[H^+]^2 + K_1[H^+] + K_1 K_2}$$

Derivation of Equation (7)

$d[P]/dt = -d[1]_T/dt = (k_b + k_2^{obs,trans}[trans]_T + k_2^{obs,cis}[cis]_T)[1]_T$

Since $[trans]_T = 0.29[RSH]_T$ and $[cis]_T = 0.71[RSH]_T$

$d[P]/dt = \{k_b + (0.29k_2^{obs,trans} + 0.71k_2^{obs,cis})[RSH]_T\}[1]_T$

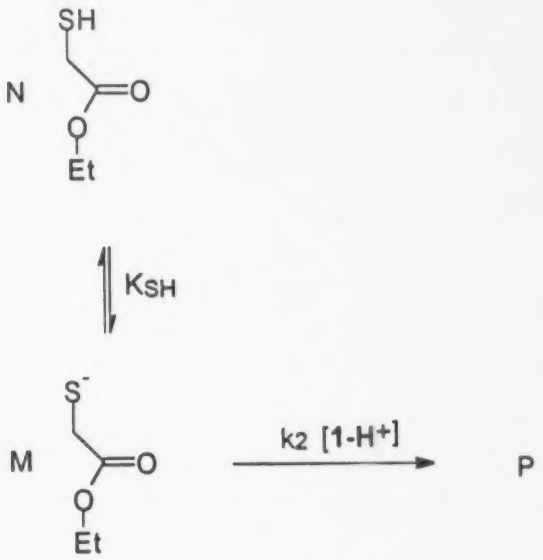
$= \{k_b + (k_2^{obs,cis/trans})[RSH]_T\}[1]_T$

so that

$$(7) \qquad k_2^{obs,cis/trans} = 0.29k_2^{obs,trans} + 0.71k_2^{obs,cis}$$

Derivation of Equation **(11)** for the process shown in **Scheme 18**

Scheme 18:



$d[P]/dt = k_b[\mathbf{1}]_T + k_2[M][\mathbf{1}\text{-}H^+]$

$= (k_b + k_2\alpha_M\alpha_{1\text{-}H^+}[RSH]_T)[\mathbf{1}]_T$

$= (k_b + k_2^{obs}[RSH]_T)[\mathbf{1}]_T$

where $k_2^{obs} = k_2\alpha_M\alpha_{1\text{-}H^+}$

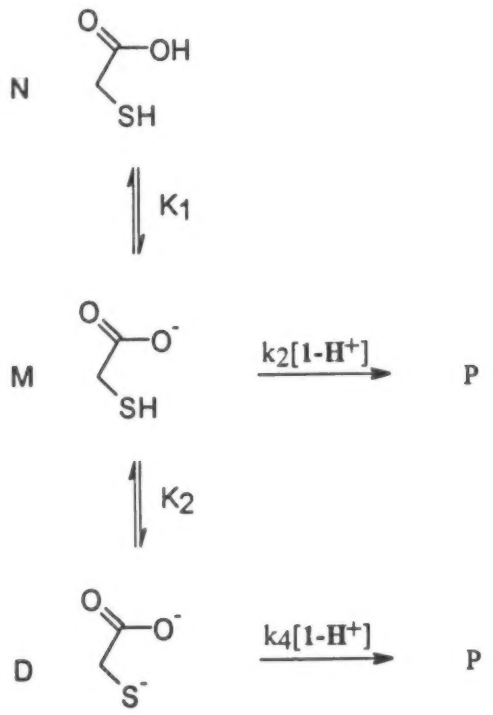
$\alpha_M = K_{SH}/(K_{SH} + [H^+])$; $\alpha_{1\text{-}H^+} = [H^+]/(K_A' + [H^+])$

Substituting these equations into the expression for k_2^{obs} and making the approximation $K_A' + [H^+] \approx K_A'$ then gives:

(11) $$k_2^{obs} = \frac{k_2 K_{SH}[H^+]}{K_A'(K_{SH} + [H^+])}$$

Derivation of Equation (13)

For the process shown below:



$d[P]/dt = k_b[\mathbf{1}]_T + k_2[M][\mathbf{1}\text{-}H^+] + k_4[D][\mathbf{1}\text{-}H^+]$

$= \{k_b + (k_2\alpha_M\alpha_{1\text{-}H^+} + k_4\alpha_D\alpha_{1\text{-}H^+})[RSH]_T\}[\mathbf{1}]_T$

$= (k_b + k_2^{obs}[RSH]_T)[\mathbf{1}]_T$

where $k_2^{obs} = k_2\alpha_M\alpha_{1\text{-}H^+} + k_4\alpha_D\alpha_{1\text{-}H^+}$

$\alpha_M = K_1[H^+]/([H^+]^2 + K_1[H^+] + K_1K_2)$; $\alpha_D = K_1K_2/([H^+]^2 + K_1[H^+] + K_1K_2)$

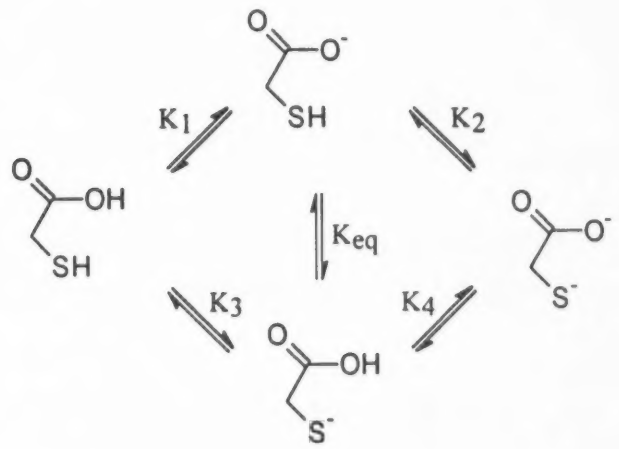
$\alpha_{1\text{-}H^+} = [H^+]/(K_A' + [H^+])$ (See Derivation of Equation (5))

Substituting these equations into the expression for k_2^{obs} and making the approximation $K_A' + [H^+] \approx K_A'$ then gives:

$$\textbf{(13)} \quad k_2^{obs} = \frac{\left(k_2 K_1 \left[H^+\right]^2 / K_A^{'}\right) + \left(k_4 K_1 K_2 \left[H^+\right] / K_A^{'}\right)}{\left[H^+\right]^2 + K_1\left[H^+\right] + K_1 K_2}$$

Estimation of K_{eq} in Equation **(14)** for thioglycolic acid

K_{eq} in equation **(14)** can be estimated by analysis of the thermodynamic cycle below:



Here, K_1 and K_2 represent the thermodynamic pK_A's for thioglycolate ionization.

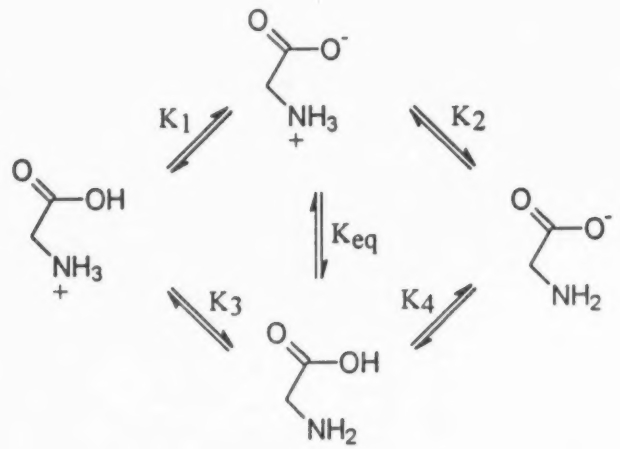
$K_{eq} = K_3/K_1$

$K_1 = 2.82 \times 10^{-4}$

K_3 can be estimated as the ionization constant for $HSCH_2COOEt$[46]; $K_3 \approx 1.0 \times 10^{-8}$

Therefore $K_{eq} \approx 1.0 \times 10^{-8}/2.82 \times 10^{-4} = 3.5 \times 10^{-5}$

Estimation of K_{eq} in equation (15) for glycine



Here K_1 and K_2 are the thermodynamic pK_A's for glycine ionization.

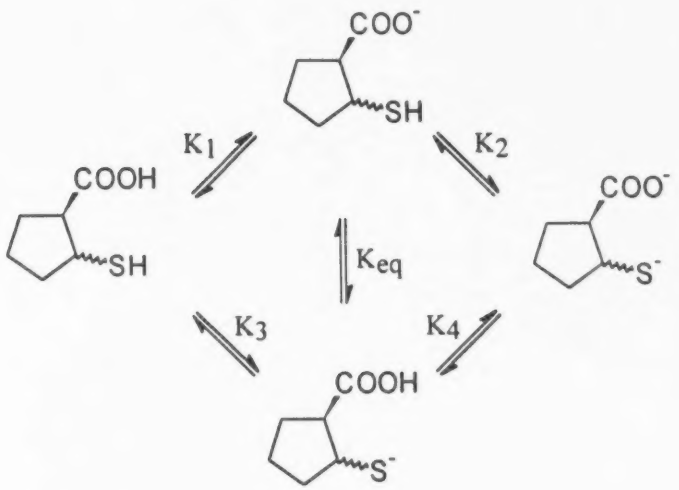
$K_{eq} = K_3/K_1$

$K_1 = 4.57 \times 10^{-3}$

K_3 can be estimated as the ionization constant for H_3N^+-CH_2-COOMe[57] ; $K_3 \approx 1.58 \times 10^{-8}$

Therefore $K_{eq} \approx 1.58 \times 10^{-8}/4.57 \times 10^{-3} = 3.5 \times 10^{-6}$

Estimation of K_{eq} in equation **(18)** for cis and trans 2-mercaptocyclopentane carboxylic acids.



(In the case of these thiol acids both K_1 and K_3 must be estimated since the thermodynamic pK_A's for these molecules have not been measured. As a result the value for K_{eq} obtained from this analysis should be viewed as only a rough estimate.)

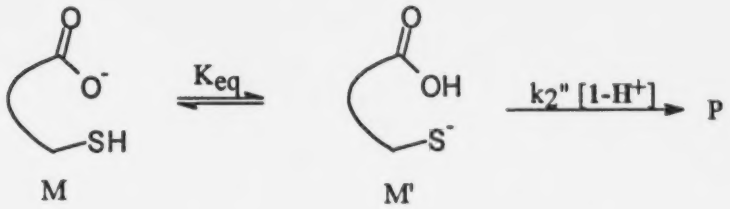
K_1 can be estimated as the first ionization constant for 3-mercaptopropionic acid ($HS\text{-}CH_2\text{-}CH_2\text{-}COOH$)[45]; $K_1 \approx 4.17 \times 10^{-5}$.

K_3 can likewise be estimated as the ionization constant for $HS\text{-}CH_2\text{-}CH_2\text{-}COOEt$[58]; $K_3 \approx 3.24 \times 10^{-10}$.

Therefore $K_{eq} = K_3/K_1 \approx 3.24 \times 10^{-10}/4.17 \times 10^{-5} = 7.77 \times 10^{-6}$

Calculation of rate constants for attack of the thiolate form of the monoanionic thiol acids on 1-H^+

For the following process:



$d[P]/dt = k_2''[M'][\mathbf{1\text{-}H^+}]$

$= k_2''K_{eq}[M][\mathbf{1\text{-}H^+}]$

Therefore $k_2''K_{eq}$ can be equated to k_2 obtained from NLLSQ fitting of equation **(5)** (thioglycolic acid) or from linear regession fitting of equation **(9)** (cyclopentane thiol-acids). The values of K_{eq} already determined for thioglycolic acid and for cis and trans 2-mercaptocyclopentane carboxylic acids (Appendix 2) can then be used to calculate k_2''.

Thioglycolic acid: $k_2'' = k_2/Keq \approx 1.9\times10^4/3.55\times10^{-5} = 5.3\times10^8\ M^{-1}s^{-1}$

Cis 2-mercaptocyclopentanecarboxylic acid: $k_2'' \approx 4.3\times10^4/7.77\times10^{-6}$

$= 5.5\times10^9\ M^{-1}s^{-1}$

Trans 2-mercaptocyclopentanecarboxylic acid: $k_2'' \approx 2.2\times10^9\ M^{-1}s^{-1}$

X-ray Crystal Structure of *trans* 2-S-Benzoylcyclopentanecarboxylic Acid (**20**)

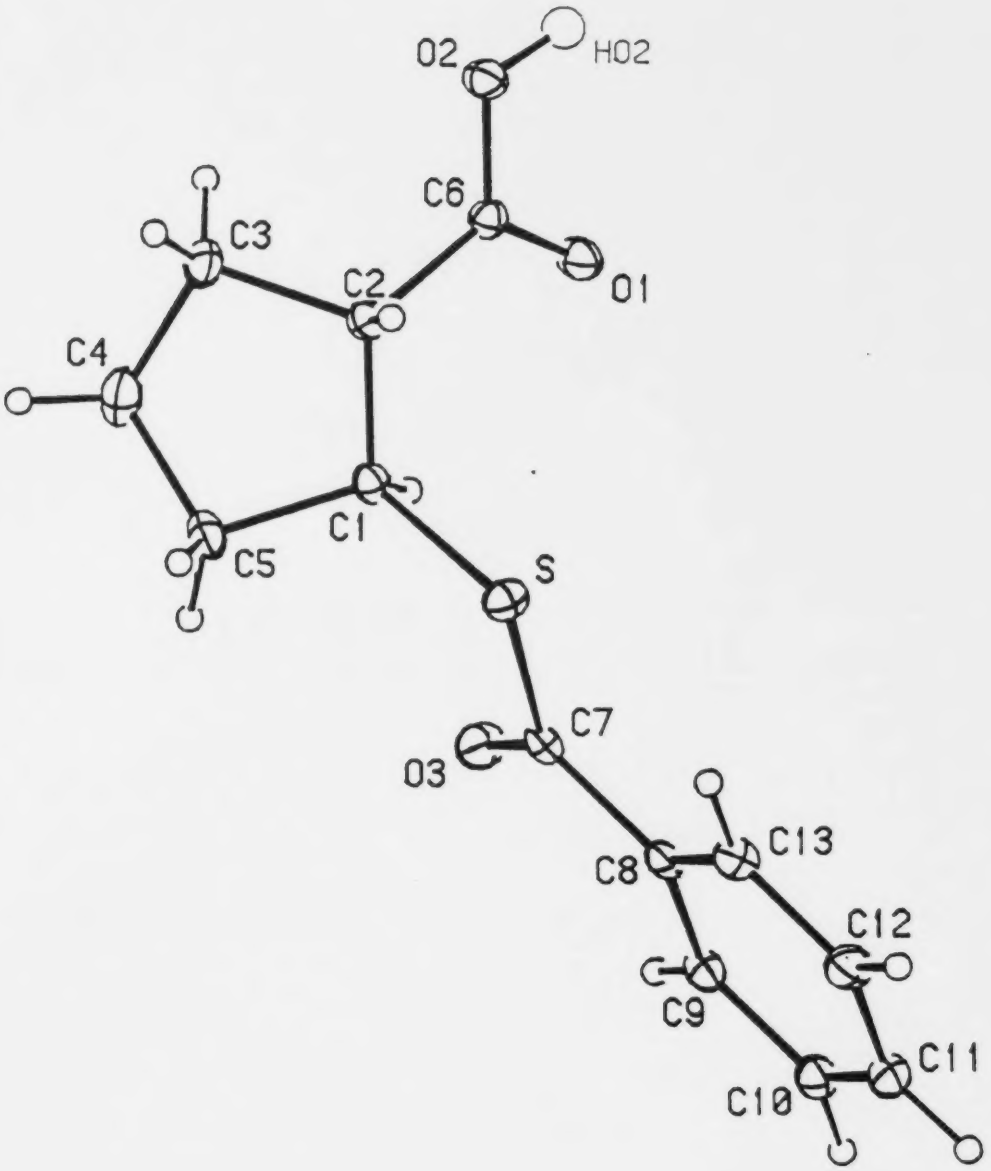


Table A-1 Crystallographic Experimental Details

A. Crystal Data	
formula	$C_{13}H_{14}O_3S$
formula weight	250.32
crystal dimensions (mm)	0.51 × 0.36 × 0.30
space group	$P\bar{1}$ (No. 2)
unit cell parameters	
a (Å)	5.8729 (6)
b (Å)	10.293 (1)
c (Å)	10.889 (1)
α (deg)	111.40 (1)
β (deg)	99.307 (9)
γ (deg)	95.06 (1)
V (Å^3)	597.0 (3)
Z	2
ρ_{calcd} (g cm^{-3})	1.392
μ (cm^{-1})	2.52
B. Data Collection and Refinement Conditions	
diffractometer	Enraf-Nonius CAD4
radiation (λ [Å])	Mo Kα (0.71073)
monochromator	incident beam, graphite crystal
temperature (°C)	–50
take-off angle (deg)	3.0
detector aperture (mm)	(3.00 + tanθ) horiz × 4.00 vert

(continued)

Table A-1 Crystallographic Experimental Details (continued)

crystal-to-detector distance (mm)	173
scan type	θ–2θ
scan rate (deg min^{-1})	6.7–1.3
scan width (deg)	$0.50 + 0.344\tan\theta$
data collection 2θ limit (deg)	50.0
total data collected	2212 ($\pm h$ $\pm k$ $+l$)
range of absorption correction factors	0.8459–1.0936
total unique data	2091
number of observations (NO)	1803 ($I > 3\sigma(I)$)
final no. parameters varied (NV)	158
R^a	0.033
$R_w{}^b$	0.050
GOF^c	1.865

$^aR = \Sigma||F_o|-|F_c||/\Sigma|F_o|$.

$^bR_w = [\Sigma w(|F_o|-|F_c|)^2/\Sigma wF_o^2]^{1/2}$.

^{c}GOF $= [\Sigma w(|F_o|-|F_c|)^2/(NO-NV)]^{1/2}$.

Table A-2 Selected Interatomic Distances (Å)

Atom1	Atom2	Distance	Atom1	Atom2	Distance
S	C1	1.803 (1)	C3	C4	1.529 (2)
S	C7	1.771 (1)	C4	C5	1.512 (2)
O1	C6	1.217 (1)	C7	C8	1.491 (2)
O2	C6	1.320 (2)	C8	C9	1.387 (2)
O2	HO2	0.89 (2)	C8	C13	1.391 (2)
O3	C7	1.215 (2)	C9	C10	1.384 (2)
C1	C2	1.536 (2)	C10	C11	1.390 (2)
C1	C5	1.524 (2)	C11	C12	1.379 (2)
C2	C3	1.555 (2)	C12	C13	1.388 (2)
C2	C6	1.502 (2)			

Table A-3 Selected Interactomic Angles (deg)

Atom1	Atom2	Atom3	Angle	Atom1	Atom2	Atom3	Angle
C1	S	C7	100.42 (6)	O2	C6	C2	113.3 (1)
C6	O2	HO2	109 (1)	S	C7	O3	122.2 (1)
S	C1	C2	111.16 (8)	S	C7	C8	115.37 (9)
S	C1	C5	114.5 (1)	O3	C7	C8	122.4 (1)
C2	C1	C5	104.1 (1)	C7	C8	C9	118.5 (1)
C1	C2	C3	105.0 (1)	C7	C8	C13	122.1 (1)
C1	C2	C6	114.1 (1)	C9	C8	C13	119.5 (1)
C3	C2	C6	112.8 (1)	C8	C9	C10	120.4 (1)
C2	C3	C4	106.0 (1)	C9	C10	C11	120.1 (1)
C3	C4	C5	104.5 (1)	C10	C11	C12	119.6 (1)
C1	C5	C4	102.9 (1)	C11	C12	C13	120.5 (1)
O1	C6	O2	122.5 (1)	C8	C13	C12	119.9 (1)
O1	C6	C2	124.2 (1)				

Table A-4 Atomic Coordinates and Equivalent Isotropic Displacement Parameters

Atom	*x*	*y*	*z*	B_{eq}, Å^2
S	0.28694(7)	0.30093(4)	0.43074(4)	2.64(1)*
O1	0.3201(2)	0.0750(1)	0.0952(1)	3.25(3)*
O2	0.6311(2)	0.1866(1)	0.0612(1)	2.82(3)*
O3	-0.1687(2)	0.2221(1)	0.3679(1)	3.33(3)*
C1	0.2053(3)	0.3272(2)	0.2756(2)	2.31(4)*
C2	0.4142(3)	0.3282(2)	0.2073(1)	2.38(4)*
C3	0.3696(4)	0.4274(2)	0.1302(2)	3.95(5)*
C4	0.1433(4)	0.4823(2)	0.1612(2)	4.08(5)*
C5	0.1239(3)	0.4682(2)	0.2927(2)	3.13(4)*
C6	0.4488(3)	0.1840(2)	0.1176(1)	2.28(4)*
C7	0.0101(3)	0.2350(2)	0.4480(2)	2.33(4)*
C8	0.0125(3)	0.1935(1)	0.5658(1)	2.23(4)*
C9	-0.1966(3)	0.1341(2)	0.5816(2)	2.63(4)*
C10	-0.2011(3)	0.0904(2)	0.6874(2)	3.00(4)*
C11	0.0044(3)	0.1052(2)	0.7783(2)	3.14(4)*
C12	0.2122(3)	0.1652(2)	0.7633(2)	3.19(5)*
C13	0.2179(3)	0.2091(2)	0.6574(2)	2.74(4)*
HO2	0.647(4)	0.098(2)	0.014(2)	2.6(5)

Anisotropically-refined atoms are marked with an asterisk (*). Displacement parameters for the anisotropically refined atoms are given in the form of the equivalent isotropic Gaussian diplacement parameter, B_{eq}, defined as $^4/_3[a^2\beta_{11} + b^2\beta_{22} + c^2\beta_{33} + ab(\cos\gamma)\beta_{12} + ac(\cos\beta)\beta_{13} + bc(\cos\alpha)\beta_{23}]$.

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